REMARKS/ARGUMENTS

Petition is hereby made under the provisions of 37 CFR 1.136(a) for an extension of two months of the period for response to the Office Action. Authorization to charge the prescribed fee to our deposit account is enclosed.

The courtesy of the Examiner in granting an Interview to the applicants representative, Mr. Michael Stewart, is much appreciated. It is believed that the Interview was material in advancing the prosecution of this application. The remarks made herein complement and supplement to those made to the Examiner at the Interview.

As discussed at the Interview, claims 19 to 25 have been deleted to reduce the issues for consideration in this case. The deletion of such claims is made without prejudice to the applicants right to file a continuation application directed thereto.

The Examiner objected to claim 52 as containing a typographical error. The term "bout" has been deleted from claim 52.

The Examiner rejected claims 19, 20, 22 to 26 and 28 to 54 under 35 USC 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The objections with respect to claims 19, 20 and 22 to 25 are moot as these claims have been deleted.

With respect to the remaining claims, in essence, two objections are raised, the first with respect to antecedent basis for certain terminology found in the claims. Claims 26, 29, 30, 41 and 50 have been amended to provide proper antecedent language for the terms in question.

The second rejection pertained to the metes and bounds of certain terms recited in claims 26, 51 and 52. The term "about" has been deleted from these claims.

Having regard to the deletion of claims 19 to 25 and the amendments made to the claims, it is submitted that the claims can no longer be considered to be indefinite and hence the rejection of claims 19, 20, 23 to 26 and 28 to 54, insofar as they remain in the application and in their amended form, under 35 USC 112, second paragraph, as being indefinite, should be withdrawn.

The Examiner rejected claims 19, 20, 22 to 26 and 28 to 54 under 35 USC 103(a) as being unpatentable over Murray (US 6,005,076) in view of Jones et al (US 4,158,656) and Jones et al (US 6,146,669) and further in view of Diosady et al (US 2003/0060607), Maenz et al (US 6,800,708) and Holbrook et al (US 6,132,795). Reconsideration is requested having regard to the amended form of the claims and the remarks herein.

With the deletion of claims 19 to 25, there remains a single independent claim in this application, namely claim 26. Claim 26 defines a process for preparing a canola protein isolate of improved colour from a canola oil seed meal which comprises sequentially-recited steps.

The canola oil seed meal is extracted to cause solubilization of canola protein in the canola oil seed meal to form an aqueous canola protein solution having a pH of about 5 to about 6.8. The concentration of protein in the aqueous canola protein solution is increased while the ionic strength of the aqueous canola protein solution is maintained substantially constant by effecting ultrafiltration of the aqueous canola protein solution to provide a concentrated canola protein solution.

The concentrated canola protein solution then is subjected to diafiltration using about 2 to about 20 volumes of diafiltration solution, until no

significant further quantities of phenolics and colour are present in the permeate from the diafiltration operation.

Thereafter, the diafiltered canola protein solution is diluted into chilled water having a temperature below 15°C to form discrete canola protein micelles in the chilled water. The canola protein micelles aare settled to form an amorphous, sticky, gelatinous, gluten-like canola protein micellar mass which is recovered from supernatant. The canola protein micellar mass has a protein content of at least 90 wt% (N x 6.25) on a dry weight basis.

The key feature of this claim which distinguishes it from the prior art is the combination of an ultrafiltration step on the aqueous canola protein solution to concentrate the same followed by a diafiltration step on the concentrated canola protein solution to remove phenolics and colour.

As set forth in the disclosure, one difficulty with canola protein isolates is a relatively dark colour and an undesirable flavour, inhibiting their use in certain food applications. Phenolic compounds have been reported to be responsible for these problems of canola protein products, including canola oil seed meal. Canola contains about ten times the quantity of phenolic compounds as is found in soybean and may comprise sinapine and condensed tannins. Upon oxidation, phenolic compounds can give raise to the development of a dark colour.

The colour problem is particularly acute with canola protein products produced by isoelectric precipitation where strongly alkaline conditions lead to ready oxidation of phenolic compounds to quinines, which then react with the protein and impart a dark green or brown colour to the protein and solutions thereof.

The procedure of the present invention serves to remove phenolics and colour during the processing of the aqueous canola protein solution produced by extraction of the canola oil seed meal to form the canola protein micellar mass, to

result in a canola protein isolate of improved colour, which then may be used in a wide range of food applications.

The Murray reference generally describes a process for the preparation of an oil seed protein isolate, including a canola protein isolate, in which a canola protein micellar mass is formed. The reference describes the steps of extracting the oil seed meal to form an aqueous protein solution, concentrating the aqueous protein isolate, diluting the concentrated protein solution, and settling the micelles to form a protein micellar mass.

However, the Murray reference lacks disclosure of the combination of the sequential steps of:

- increasing the canola protein concentration of the aqueous canola protein solution while maintaining the ionic strength substantially constant by the use of a selective membrane technique to provide a concentrated canola protein solution,

- subjecting the concentrated canola protein solution to diafiltration using about 2 to about 20 volumes of diafiltration solution, until no significant further quantities of phenolics and colour are present in the permeate.

It is noted that claim 26 utilizes the recitation of "the sequential steps of" to emphasize that these steps are effected in the recited order.

The Examiner points to Murray as disclosing a diafiltration step in col. 7, lines 45 to 50 followed by a concentration step. The Examiner also referred to col. 7, lines 10 to 15. This latter passage refers to concentration of the defatted protein solution while maintaining the ionic strength thereof substantially constant to form a concentrated defatted protein solution.

Applicants claims are directed to producing a canola protein isolate of improved colour. Murray is wholly silent as to any procedure which results in improved colour. The Examiner points to col. 5, lines 12 to 22 for the statement:

> "As is well known, ultrafiltration and similar selective membrane techniques permit low molecular weight species to pass therethrough while preventing higher molecular weight species from so doing. The low molecular weight species include not only the ionic species of the food grade salt but also low molecular weight materials extracted from the source material, such as, carbohydrates, pigments etc. The molecular weight cut-off of the membrane is usually chosen to ensure retention of substantially all of the proteins in the solution."

To the extent that Murray discloses the steps of diafiltration and concentration by ultrafiltration, the reference recites:

> "The supernatant was diafiltered and then concentrated on a membrane with a molecular weight cut-off rated at 30,000 daltons."

Thus, this procedure effects first diafiltration and second ultrafiltration. According to claim 26, applicants first effects concentration by ultrafiltration and then colour removal by diafiltration. The latter step is effected using 5 to 20 volumes of diafiltration solution until no significant quantities of phenolics and visible colour are present in the permeate. The Murray reference is silent as to any particular conditions for diafiltration.

Jones et al (US 4,158,656) discloses the canola colour problem referred to above. In Jones et al, a canola oil seed meal is extracted with an aqueous alcohol solvent solution under non-oxidizing conditions to extract phenolics and colour from the canola oil seed meal to form a protein concentrate (not an isolate, as required by applicants claims). Jones et al (US 4,158,656) is silent as to any procedure in which aqueous canola protein solution extracted from canola oil seed meal is concentrated, diafiltered and then diluted to form a protein micellar mass, as required by claim 26.

Jones et al (US 6,146,669) apparently is relied on solely for the teaching that, in a process that involves the incubation of a protein - containing material in a culture medium that contains oilseed-based material, it is typically advisable to pasteurize the material to ensure that microbial activity is minimized. This reference, therefore, would appear to be relevant only to claims 53 and 54.

The procedure described in Jones '669 for the preparation of an oil seed protein product is wholly different from applicants process of preparing a canola protein isolate. There would appear to be nothing in the Jones '669 reference which would cause a person skilled in the art to modify the process described in the prior art of Murray to incorporate the pasteurization step claimed in claims 53 and 54.

The Diosady et al reference is concerned with the production of high quality canola protein isolates. The Examiner refers to a specific teaching that, in Example 2, col. 23, lines 10 to 15, insoluble PVP is added to treat the solution for one hour and then is separated by filtration.

In the procedure described in Diosady, in Example 2, canola oil seed meal is extracted with sodium hydroxide solution in the presence of sodium sulphite and the residual meal is separated by centrifugation and the supernatant polished using filter paper. Following addition of NaCl and SDS, the solution is reduced in volume by ultrafiltration and then the concentrated solution is diafiltered. After the diafiltration, the solution is acidified to precipitate what is termed "precipitated protein isolate" (PPI).

The PPI is an isoelectrically precipitated product of the Diosady process, analogous to the protein micellar mass produced in applicants process and in Murray. The PVP treatment step in Diosady is applied to the solution following separation from the PPI. The PVP treatment step in Diosady is not effected prior to separation of the protein isolate but rather subsequently and would be considered to

be the equivalent of the treatment of the supernatant from the protein micellar mass produced herein and in the Murray reference. It would appear that this reference is relevant only with respect to claims 46 to 49.

Accordingly, there is no teaching in Diosady which suggests treatment of the diafiltered concentrated canola protein solution with PVP or other colour-adsorbing agent, prior to dilution to form the protein micelles.

Maenz is concerned with a process for the aqueous extraction, fractionation and enzymatic treatment of oil seed proteins. The Examiner refers specifically to col. 3, ll 55 to 61 for the teaching of ultrafiltration and diafiltration. The passage refers to a literature reference said to disclose the processing of a liquid phase from the separation of a canola protein isolate prepared by isoelectric precipitation. A copy of the complete literature reference is enclosed for the Examiner's convenience.

As can be seen, Diosady is a co-author of the paper. As with the PVP treatment described in Diosady, the ultrafiltration and diafiltration steps are carried out on a liquid material which is the equivalent of applicants supernatant from the precipitation of protein micellar mass and, therefore, only relevant to claims 38 to 45.

The Holbrook et al reference teaches a vegetable protein composition. The Examiner points to col. 5 for a teaching that the vegetable protein concentrate or isolate is an alcohol extracted or washed material and to cols. 8 and 9 that the protein can be canola protein. This reference would appear to be relevant only to claim 37.

Accordingly, it is submitted that all pending claims are patentable over the applied prior art and hence it is submitted that the rejection of claims 19, 20, 22 to 26 and 28 to 54, insofar as they remain in the application and in their amended form, under 35 USC 103(a) as being unpatentable over Murray (US 6,005,076) in view of Jones et al (US 4,158,656) and Jones et al (US 6,146,689) and further in

view of Diosady et al (US 2003/0060607), Maenz et al (US 6,800, 308) and Holbrook et al (US 6,132,795), should be withdrawn.

It is believed that this application is now in condition for allowance and early and favourable consideration and allowance are respectfully solicited.

Respectfully submitted,

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REVIEWS

Nutritional Value of Proteins from Different Food Sources. A Review

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The nutritional value or quality of structurally different proteins varies and is governed by amino acid composition, ratios of essential amino acids, susceptibility to hydrolysis during digestion, source, and the effects of processing. To optimize the biological utilization of proteins, a better understanding is needed of the various interrelated parameters that influence their nutritive value. This review attempts to contribute to this effort. It discusses methods used for protein quality evaluation, research needs to facilitate labeling foods for protein quality, and factors influencing protein quality including amino acid analysis, digestion, food processing, antinutrients, and protein-energy relationships. Recent studies on the nutritional quality of more than 50 common and uncommon protein sources including cereals, legumes, other seeds, meat, seafood, insects, leaves, mushrooms, and potatoes are reviewed. Also described are advantages of consuming low-quality proteins fortified with essential amino acids, nutritional benefits of mixtures of complementary protein sources, plant genetic approaches to improving the nutritive value of foods, problems associated with liquid diets for adults and infants, socioeconomic aspects of new protein foods, and the influence of protein type and quality on lactation, the immune system, and serum lipids. This integrated overview is intended to stimulate interest in the introduction and use of new protein sources for feeding the ever-growing world population.

Keywords: *Amino acids; digestibility; food protein sources; health; malnutrition; mixed proteins; nutritional quality; protein quality*

INTRODUCTION

Proteins are an essential component of the diet needed for survival of animals and humans. Protein's basic function in nutrition is to supply adequate amounts of needed amino acids. The protein quality, also known as the nutritional or nutritive value of a food, depends on its amino acid content and on the physiological utilization of specific amino acids after digestion, absorption, and minimal obligatory rates of oxidation. Metabolism of amino acids is determined by the proportion of amino acids used for protein synthesis. Rates of oxidation of amino acids are low until the amount consumed exceeds the amount needed for protein synthesis; oxidation then increases rapidly. These observations justify the use of plasma amino acid levels and oxidation of amino acids *in vivo* to assess amino acid requirements (Block, 1989; Reichl, 1989; Scharrer, 1989; Smolin and Benevenga, 1989; Umezawa, 1989). Availability of amino acids varies with protein source, processing treatment, and interaction with other components of the diet.

As population growth continues to increase, and as the main sources of food (farms and oceans) may be approaching maximum *per capita* output, demand seems likely to outpace food production. According to the World Watch Institute (1993) (a) world grain production has fallen 8% since 1984, primarily due to decreased availability of land, water, and fertilizers; (b) both world fish and meat production per person are beginning to fall; and (c) population is increasing at a rate of 100 million per year. Expectations are that the present world population of about 5.5 billion will double by the year 2030. Famine, malnutrition, and starvation result from the inability of large proportions of the world's population to earn the means to buy food (Sen, 1993). However, similar Malthusian predictions (Hardin, 1993) that population will finally outstrip world resources have not materialized for two centuries now, presumably because food production has kept up with population growth.

The problem of obtaining sufficient protein is further compounded because, according to Zello et al. (1992), the mean daily protein requirement for young adult males of 0.6 g/kg of highly digestible, good quality protein should be increased to 0.8 g/kg. Results using an amino acid oxidation technique led them to conclude that amino acid requirements of adult humans are higher than those estimated using the N balance method. The discrepancy between results with the two types of studies has not been explained.

Table 1 shows that humans in underdeveloped countries do indeed consume more low-quality proteins compared to those living in developed countries. Table 2 lists the amino acid composition of several food sources, the amino acid requirements recommended by FAO/WHO (1991), and the higher values suggested by Young and Pellett (1991). Although the higher adult amino acid requirements can be met readily from currently recommended intakes of high-quality proteins, the results imply that the amounts of low-quality protein needed by adults to meet protein requirements may be greater than has been assumed. Piller (1991) reviews protein requirements for children. It should

Table 1. Major Sources of Protein in the Diet in Developing and Developed Countries (USDA, 1993)

source	developing (%)	developed (%)
cereals	58.8	29.1
meat	8.6	26.4
pulses	7.4	1.7
milk and dairy	5.6	16.7
fish, seafood	4.1	7.3
oil crops	3.8	1.9
vegetables	3.5	3.5
starchy roots	3.1	3.2
eggs	1.6	4.3
offals	1.2	2.2
fruit	1.0	1.1

also be emphasized that the critical importance of low energy intakes, which reduce the efficiency of utilization of proteins, is a major cause of worldwide protein malnutrition (Olson, 1975). If the major problem in the occurrence of malnutrition is inadequate energy (food) intake, emphasis should be on developing new food sources of high-quality protein that will yield more total energy.

This paper has the following objectives: (a) to describe techniques used to evaluate the nutritional value of food proteins; (b) to define some of the factors that increase or lessen nutritional value; (c) to review the widely scattered literature on the nutritional value of a variety of food sources; and (d) to suggest research needs in the hope of stimulating interest in introducing new food sources into the food chains of many nations and optimizing health benefits of proteins.

METHODS FOR EVALUATING PROTEIN QUALITY

The concept of essentiality of amino acids underlies all protein quality methods. According to Mercer et al. (1989), amino acids provide essential nitrogen for the synthesis of protein and other biological molecules. They may be divided into three categories based on their absolute or relative rates of protein synthesis *in vivo*: (a) *indispensable*—histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine; (b) *conditionally indispensable*—arginine, cysteine, tyrosine; (c) *dispensable*—alanine, asparagine, aspartic acid, glutamine, glutamic acid, glycine, proline, serine.

Laidlaw and Kopple (1987) reviewed newer ideas on the indispensability of amino acids for promoting growth in the young, preventing disease, and maintaining a positive nitrogen balance in the young and old. They cite evidence for the indispensability of (a) taurine in infants and cats; (b) cysteine in preterm infants, older children with metabolic disorders, and malnourished patients suffering from compromised liver function such as cirrhosis; (c) tyrosine in premature infants and in the malnourished or the elderly; and (d) basic amino acids such as arginine, citrulline, and ornithine that are involved in the urea cycle (Millward, 1994; Young, 1994).

The subject of indispensable amino acids is also critically examined by Dillon (1992) and Heger and Frydrych (1989). The related concept of subdividing amino acid requirements for growth and maintenance is discussed by Owens and Pertigrew (1989).

Protein quality depends on the concentration and ratios of constituent amino acids making up a specific protein. The greater the ratio of indispensable amino acids, the greater the biological value or quality. The distribution of a specific amino acid within this ratio is also of paramount importance. Proteins that are deficient in one or more amino acids are of poor quality. For example, tryptophan and lysine are limiting in corn, lysine in wheat and other cereals, and methionine in soybeans and other legumes. The objective of all methods for evaluating the adequacy of the protein component of a human diet is to estimate the amount of biologically utilizable protein. Such estimates depend on the total amount consumed and its quality.

Efforts to accurately measure protein nutritional quality started at the beginning of this century. These efforts are being continuously extended with improved approaches, methodology, and definitions. The following references offer detailed critical discussions of advantages and limitations of a variety of proposed techniques: Benevenga et al. (1994); Davis and Harris (1989); FAO/WHO, (1991); Friedman (1974, 1975a,b, 1977a,b, 1978a,b, 1984, 1986, 1989, 1991); Grasset (1991); Hegsted (1977); Hermus (1993); Hurt et al. (1978); Matthews (1991); McLaughlan and Keith (1978); Pellett and Young (1980); Sarwar et al. (1984, 1985, 1989). The following brief definitions give an indication of some of the more widely used techniques to measure protein nutritional quality (Friedman, 1978a; FAO/WHO, 1991).

Amino Acid Availability % = (the total intake of amino acid − fecal excretion of amino acid)/(total intake of amino acid) × 100.

Amino Acid or Chemical Score = (mg of amino acid in 1 g of a test protein)/(mg of amino acid in a reference protein).

Digestibility, True = [N consumed (test animals) − N excreted in feces (test group) + metabolic N in feces]/N consumed (test animals) × 100, where metabolic N in feces = N excreted in feces (protein-free animals) × food intake (test group)/food intake (protein-free animals) (see also Nitrogen Balance Methods).

Limiting Amino Acid = an essential amino acid in a protein that shows the greatest difference in concentration from the same amino acid in a reference, high-quality protein.

Net Protein Ratio (NPR) = (weight gain of test animals + weight loss of animals fed non-protein basal diet)/(weight of protein consumed).

Net Protein Utilization (NPU) = (N retained/N intake) × 100.

Table 2. Essential Amino Acids of Different Protein Sources (Sarwar et al., 1983) and FAO/WHO (1991) and Young and Pellett (1991) Suggested Human Amino Acid Requirements (Milligrams per Gram of Protein)

						FAO/WHO			Young and Pellett
amino acid	ANRC casein	beef	egg white	soy protein	wheat flour	1 yr old	2–5 yr old	10–12 yr old	adult	preschool–adult
Thr	46.4	42.1	46.8	38.4	29.3	43	34	28	9	25
Cys + Met	34.9	32.7	66.4	68.1	38.7	42	25	22	17	25
Val	68.5	45.4	67.8	49.1	42.7	55	35	25	13	35
Ile	53.6	41.8	52.8	47.1	33.4	46	28	28	13	35
Leu	101.6	77.5	87.6	85.1	68.5	93	66	44	19	65
Tyr + Phe	125.4	70.2	90.8	96.6	77.8	72	63	22	19	65
His	29.7	32.0	22.5	25.4	21.9	20	19	19	16	–
Lys	84.4	79.4	69.8	63.4	26.6	66	58	44	16	50
								9	5	10

Table 3. Ranking of Diets by Five Different Protein Quality Indices (Mean Values from Six Laboratories) [Adapted from Sarwar et al. (1984)]

diet	PER	NPR	NU	RPER	RNPR
ANRC casein + 0.1% Met	4.04	5.30	5.68	100	100
egg white solids	3.71	5.08	5.43	91	95
minced beef	3.36	4.83	5.16	83	91
rapeseed protein concentrate (RPC)	3.29	4.59	4.90	81	87
ANRC casein	3.13	4.55	4.83	78	86
pea flour + 0.1% Met	2.91	4.33	4.59	72	81
whole wheat flour + ANRC casein[a]	3.01	4.20	4.47	74	79
whole wheat flour + minced beef[a]	2.97	4.16	4.41	73	79
whole wheat flour + egg white solids[a]	2.90	4.09	4.36	72	77
soy protein + 0.1% Met	2.55	3.72	3.87	64	70
whole wheat flour + pea flour[a]	2.18	3.50	3.60	54	66
whole wheat flour + soy protein[a]	2.21	3.37	3.53	55	64
whole wheat flour + RPC[a]	2.08	3.34	3.49	51	63
whole wheat flour + 0.16% Lys	1.70	2.91	2.98	42	53
pea flour	1.56	2.88	2.89	39	54
soy protein	1.60	2.74	2.79	30	51
whole wheat flour	0.95	2.35	2.29	23	44

[a] 50/50 mixture based on protein content.

Nitrogen Balance Methods: Apparent digestibility coefficient (ADC) = 100 $(I - F)/I$; net protein utilization (NPU) = 100 $[I - (R_0 - B_0)]/I$; nitrogen balance (B) = $I - (F + U)$; nitrogen balance index (NBI) = 100 $(B - B_0)/I$; true absorption (A) = $I - (F - F_0)$; biological value (BV) = 100 $(B - B_0)/A$; true digestibility coefficient (TDC) = 100 $[I - (F - F_0)]/I$, where I, I_0 = N intake of animals with and without proteins, respectively; F, F_0 = N in feces of animals fed with and without proteins, respectively; U = N in urine; B_0 = N balance of animals fed without proteins; R, R_0 = N of whole animals fed with and without proteins, respectively.

Nitrogen Conversion Factor = numerical factor used to convert the nitrogen content of a specific food to the protein content; typically ranges from 5.18 to 6.38, generally 6.25. Data should be reported on a dry basis along with the conversion factors used (Simmonds, 1995).

Nitrogen Requirement for Humans = endogenous N in urine (U) + endogenous N in feces (F) + N lost as sweat, skin, and integument (S) + N required for growth (G).

Plasma Amino Acid Ratio (PAA) = the relative changes in concentration of each of the free essential amino acids in plasma after consumption of a protein food, expressed as a function of the specific amino acid requirements of an animal or human.

Protein Efficiency Ratio (PER) = (weight gain of a test group)/(total protein consumed).

PER, Adjusted, Corrected = (PER of a test protein)/(PER of a casein control).

PER, Relative (RPER) = (PER of test animal)/(PER of reference animal) × 100.

Relative Nutritive Value (RNV) = (weight gain of a test group) + 0.1(initial + final weights of the test group)/[N consumed relative to a standard protein (lactalbumin)].

Reference Protein = a protein of high biological value such as lactalbumin containing a specified pattern of amino acids.

Generally, a PER below 1.5 approximately describes a protein of low or poor quality; between 1.5 and 2.0, an intermediate quality; and above 2.0, good to high-quality. Many studies use adjusted PER, where the actual PER is adjusted for the PER observed for casein, assuming a standard casein PER of 2.5. Temler et al. (1983) found a linear relation between weight gain, [illegible] for 12 food sources. The highest value for RNV equals 1.0. For BV and NPU, 100% denotes the highest quality protein.

The methods listed provide two types of information. Most of the methods, mainly animal assays, can be used to assess damage to proteins during processing and to rank foods according to their protein quality, as shown in Table 3. These methods may not be useful for quantitatively estimating the effectiveness of foods in meeting human protein needs or for meeting nutritional labeling requirements. This type of information can be obtained with methods based on measurements of amino acid composition and digestibility.

Because of the current emphasis on the need for labeling of foods for both quantity and nutritional quality of protein and because many food proteins are adversely affected by food processing (Friedman, 1991), further development of ideal methods for protein quality evaluation is desirable. The labels of many foods now list the amount but not the quality of protein in the formulation. Because of large variations in the nutritional values of native and processed protein derived from different sources described below, the label should give at least some indication of the quality of the protein. For example, although we may know the nutritional value of wheat gluten as a pure protein, its quality often deteriorates when wheat flour is processed into bread or cereals (Friedman and Finot, 1990; Jurkovic and Colic, 1993). This issue affects the entire world population, which must consume protein to survive. Industry has resisted adopting the widely used PER method for measuring protein quality based on rat feeding studies because (a) it takes 28 days to complete; (b) it may be unreliable for some low-quality-protein foods; (c) it may not be directly relevant to human nutrition; and (d) it may be too expensive, especially when applied to food batches during the production process.

As a more accurate alternative to PER, the use of amino acid scores has been proposed (Sarwar et al., 1981, 1983, 1984). This proposal is supported by the FAO/WHO (1991). However, this method has not been widely adopted. The resistance to its use is probably due to the fact that to obtain an accurate amino acid score, one has to carry out three separate amino acid analyses—a standard analysis, one for tryptophan, and one for sulfur amino acids. Because tryptophan is destroyed by acid hydrolysis, a separate procedure must be done involving hydrolysis with base or with an acid—ninhydrin reagent (Friedman and Cuq, 1988). Because methionine may also be degraded to some extent when

the acid hydrolysis is carried out in the presence of carbohydrates, methionine plus cysteine is usually determined separately, either by oxidation with performic acid to methionine sulfone and cysteic or by some other method (Friedman et al., 1979, 1980). Moreover, total lysine values from amino acid analysis do not always reflect chemically or biologically available lysine (Carpenter, 1981; Friedman, 1982). In addition, high digestibility does not always mean high protein quality. Digestibility is a measure of protein hydrolysis and absorption of the liberated amino acids. Protein quality is a measure of the balance of the amino acids that are absorbed and utilized for growth and other purposes.

New research is needed to develop a rapid, reliable, and inexpensive assay for protein quality that meets the needs of regulatory agencies and industry (Sarwar and Peace, 1994, 1995; Young, 1995). Samman and Farias (1993) describe a 28 h method for protein nutritional quality in a variety of foods. This method is based on weight loss in rats after a 4 h fast subsequent to a 24 h feeding. They claim that their method (a) has the potential to rapidly measure changes in nutritional quality during food processing and (b) avoids the longer, more laborious, and costlier techniques used for measurement of biological value and net protein utilization.

COMPOSITION, DIGESTIBILITY, AND AVAILABILITY

Variability of Amino Acid Analyses. The amino acid composition of proteins is often used to define their nutritional quality. Cavins and Friedman (1968) recommended that the results should be presented in several forms, suitable for a variety of needs. The nitrogen recovery parameter gives a check on the analysis. The ratio of amino acids is useful in differentiating closely related proteins or highlighting decreases in specific amino acids such as lysine following chemical modifications of proteins during food processing (Eggum and Sorensen, 1989).

Sarwar et al. (1981; 1983) described inter- and intralaboratory variations in amino acid composition of several protein sources. The interlaboratory measurements of isoleucine, leucine, lysine, phenylalanine, threonine, and valine [coefficient of variation (CV) < 10%] were generally less variable than those of tryptophan (CV of 14–20%), cystine, and methionine (CV of 10–17%). Between laboratories, CV values were less than 10% for tyrosine in casein, soy protein, egg white, and minced beef but were 13% in rapeseed concentrate, 14% in wheat flour, and 16% in pea flour. For most amino acids, interlaboratory variation was less for casein, soy protein, and minced beef (CV <5–7%) than for pea flour and rapeseed concentrate (CV < 13%), while wheat flour and egg white (CV < 10%) were in the middle.

Digestion. Before a protein can serve as a nutritional source of amino acids, it must be digested. Any factor that alters digestibility would in turn affect the nutritional value of the protein (Magee and Dally, 1987). The question of how much protein must be supplied in the diet depends on the digestibility and availability of amino acids supplied by a specific diet and the ability of the consumer to respond to the amino acid supply with deposition of body protein (Fuller and Wang, 1990). Digestibility and amino acid availability are not synonymous. Rather, digestibility means the susceptibility of peptide bonds to hydrolysis, while availability refers to the chemical integrity of the amino acid, i.e. its resistance to processing by heat, high pH, oxidation, etc. ibility, especially of lysine, methionine, threonine, and tryptophan. Both parameters must be considered to define the nutritional value of proteins.

Another important consideration is that proteins are rarely consumed as the only element of a meal; rather they are eaten with other foods. The following references offer an excellent entry to the literature on parameters such as fiber, phytic acid, the nature and structure of protein, and the effects of processing that influence the susceptibility of food proteins to digestion: Begbie and Pusztal (1989); Gous (1990); Lathia and Koch (1989); Marshall et al. (1979); Nielsen (1991); Schwimmer (1981); Slump and Van Beek (1975); Stahmann and Woldegiorgis (1975).

McDonough et al. (1990), Sarwar and McDonough (1990), and Sarwar and Paquet (1989) found that (a) the apparent digestibility of crude protein varied with the concentration of protein in the diet; (b) true digestibility was independent of concentration; (c) the low digestibilities of proteins in pinto beans, kidney beans, and lentils (79–84%) were comparable to those reported earlier for beans, peas, and lentils; (d) true digestibilities for methionine, cystine, and tryptophan were up to 27% lower than values for crude protein; and (e) true digestibilities of lysine in wheat, oat, rye, and sorghum were 14% lower than those in crude protein. Lower digestibility may be due to the occurrence of amino acids in less digestible parts and to protease inhibitors, amylase inhibitors, lectins, phytates, and tannins. Oste (1991) offers an excellent summary of beneficial and adverse effects of processing on digestibilities.

Our studies (Friedman et al., 1981) show that alkali-induced racemization of amino acids and concurrent formation of lysinoalanine in food proteins can impair the nutritional quality and safety of foods by (a) decreasing the amount of essential L-amino acids, (b) decreasing digestibility and bioavailability of proteins, and (c) forming toxic products.

Finally, Saunders and Betschart (1980) suggest that incompletely digested protein from some plant sources, which may represent a considerable fraction of total protein, should be considered part of the dietary fiber composite. A similar recommendation was made by Friedman and Gumbmann (1986a) for indigestible protease inhibitor–protease complexes.

Protein–Energy Relationships. Protein–energy malnutrition (PEM) is widespread (Waterlow, 1994). The percent of children under the age of 5 who are malnourished ranges from 49.4 for Asia to 29.4 for Sub-Saharan Africa to 22.9 for the Middle East/North Africa and to 9.4 for Latin America (USDA, 1993). Since consumers rarely eat pure proteins, the question arises whether the quality of protein is affected by the energy content (caloric value) of the total diet (Carpenter, 1994; Scrimshaw and Schürch, 1992; Torun, 1988). The issue of protein–energy relationships and ideal protein–energy ratios (protein as percent of total energy) is complex. Some aspects of these relationships will be briefly summarized in this section.

Beaton (1975) suggests that protein/energy ratios are useful criteria for establishing the nutritional quality of diets. When properly used, they measure the ability of a given food to meet protein requirements if consumed in sufficient quantity to meet energy needs. At any given level of dietary protein, addition of energy improves the nitrogen balance of adults until a plateau is reached, reflecting an adequate protein intake. This beneficial effect of energy requires sufficient protein of adequate quality to permit achievement of nitrogen

of better quality. Thus, differences among proteins will appear to be greater at higher energy intakes (Callnway, 1981).

Millward (1992) points out that (a) while N balance studies may reflect differences in protein sources, there is a lack of reproducibility between studies with the same protein; (b) it is important to define two levels of protein requirements—minimum and optimum; and (c) the challenge for the future is to define upper limits of protein needs for various population groups.

Jackson (1992) points out that (a) a diet consisting of poor-quality protein may be adequate, if ingested in amounts large enough to satisfy the need for the limiting amino acid; (b) the overall protein intake is determined by the amount of energy expenditure; and (c) the level of physical activity governs protein adequacy of poor-quality proteins.

Pellet and Young (1992) point out that (a) a vast number of factors influence the relationship between protein and energy intakes and protein metabolism; (b) N balance studies are influenced by changes in food energy intakes, below or above energy needs; (c) improvement in N balance caused by increase in energy intake can be frustrated if protein intake is inadequate; similarly, the beneficial effects of an increased protein intake can be inhibited by inadequate energy intake; (d) effects of protein on energy metabolism are less significant than are effects of energy on protein (nitrogen) metabolism; and (e) a minimum percentage of protein in the diet can be specified when energy needs are met. Evidently, protein–energy relationships operate in a narrow range. One needs a given base level of protein. Above that, more energy improves nitrogen balance, but only up to a level where protein intake is adequate.

A study by Kishi et al. (1978) strikingly illustrates the impact of energy on the utilization of egg protein by young Japanese men. The subjects were fed a standard diet for 1 week followed by low-protein diets for 2 weeks. These diets contained 32, 64, and 80 mg of N/kg of whole egg. With excess energy intake, the mean caloric value was 48.2 kcal/kg. The apparent N equilibrium was found to be 82 mg of N/kg, and net protein utilization (NPU) was 56. With suboptimal energy intake (40 kcal/kg), the N requirement was 124 mg of N/kg and the NPU was 37. Thus, N balance and NPU were remarkably affected by energy intake.

The following analysis describes some problems in devising an objective index of protein–calorie malnutrition (Friedman and Orracah-Tetteh, 1978; Menefee and Friedman, 1985).

An ideal goal of nutritional research is to establish an optimal diet, i.e. a diet that could be adjusted to optimize all nutritionally related aspects of life such as health and longevity, which are of greatest importance to most of us. Unfortunately, other factors intervene. These include psychological variables, occasioned by human attraction to certain food and drink to the exclusion of other possibly more healthful or life-prolonging choices, and physical variables caused by fluctuating work and exercise habits. If one wishes to investigate optimal diets in terms of these "true" criteria, the problem may appear to be hopelessly complex. Not only are esthetic, socioeconomic, and nutritional preference factors difficult to measure, but health and longevity have meaning only over very long time frames (Nicol and Phillips, 1976).

A further complication occurs in selecting appropriate lowing are some of the more obvious ones: amount and quality of protein; amount and composition of carbohydrate and fat; mineral, vitamin, and water content; nonnutritive or antinutritive ingredients; volume and weight of ingested food; total energy intake. All of these must be adjusted for unit body weight and do not take into account such factors as age, gender, and level of activity of test subjects. Also, these variables are not independent, but rather highly interrelated. Total energy intake, for example, can be calculated from the sum of protein, carbohydrate, and fat. Optimizing such a multidimensional space is likely to be extremely difficult. As is common with biological systems, the experiments may be "sloppy"; that is, the rate and amount of food ingestion is often uncertain and hard to control, and there is little certainty about how each variable should be weighted.

Protein values based on animal studies vary with the protein content of the diet [efforts to overcome this problem are described by Dreyer and Van Der Walt (1985)]. For human needs, a more reliable indicator would be the amount of "utilizable" protein provided per unit weight, regardless of the quality. Moreover, consumption of low-quality protein along with adequate calories can, in principle, meet the needs for all of the essential amino acids. However, since dietary protein can also serve as an energy source, failure to consider dietary energy deficits can result in overestimation of possible benefits of various protein sources in ameliorating malnutrition, where diets may be deficient in energy content (Latham, 1988).

Food Processing. Processing of foods can improve nutrition, quality, safety, and taste and occasionally lead to the formation of antinutritional and toxic compounds (Finot, 1995; Friedman, 1991, 1992a). A better understanding of the molecular changes that occur during processing is needed so as to optimize beneficial effects such as quality and safety while minimizing the formation of deleterious compounds. The following is a brief summary of some results of processing.

Effect of pH and Heat. Food can deteriorate during processing and storage due to both enzymatic and Maillard-type reactions of primary amino groups with reducing sugars and other enzymatic and nonenzymatic browning reactions with nonreducing carbohydrates (Ebersdobler et al., 1991; Friedman and Molnar-Perl, 1990; Friedman et al., 1987; Kratzer et al., 1990; Oste and Friedman, 1990; Oste et al., 1991; Ziderman et al., 1989). High pH induces racemization of L-amino acid residues to D-isomers (Friedman and Gumbmann, 1984a,b, 1989) and formation of cross-linked amino acids such as lysinoalanine (Friedman and Pearce, 1989; Friedman et al., 1982a). Loss of nutritional quality of heat-treated casein is related to decreased nitrogen digestibility rather than to a simple destruction of essential amino acids (Gumbmann et al., 1983). Research is needed to differentiate between antinutritional and toxic components with the ultimate goal of minimizing or preventing the formation of such compounds.

Available Lysine. Smith and Friedman (1984) heated casein and mixtures of casein with starch, sucrose, and glucose at temperatures of 37–300 °C to model storage, autoclaving, baking/broiling, and charring. Lysine and arginine content decreased by up to 99%. The relative potencies of the three carbohydrates in affecting these changes were different at the four temperatures studied. The results show the different susceptibilities of casein to heat-induced damage in the presence of different carbohydrates (Table 4; Hurrel and

Table 4. Total and Reactive (Available) Lysine Content (LYS) and Nitrogen Recovered from the Amino Acid Analyzer for Untreated and Heat-Treated Casein and Casein−Carbohydrate Mixtures (Smith and Friedman, 1984)

material	treatment	total LYS (g/16 g of N) (from amino acid analysis)	reactive LYS (g/16 g of N) FDNB	reactive LYS (g/16 g of N) dye binding	N recovered (%)
casein	none	9.68	8.03	7.98	91.9
casein	37 °C, 10 days	9.70	7.45	7.73	92.5
casein + starch	37 °C, 10 days	9.71	7.91	7.62	91.8
casein + sucrose	37 °C, 10 days	9.24	7.39	7.17	89.7
casein + glucose	37 °C, 10 days	7.43	4.87	4.54	65.5
casein	121 °C, 1 h	9.40	7.83	7.38	88.8
casein + starch	121 °C, 1 h	9.50	7.83	7.42	85.8
casein + sucrose	121 °C, 1 h	2.73	1.05	0.81	88.4
casein + glucose	121 °C, 1 h	3.15	1.54	1.95	58.8
casein	200 °C, 1 h	8.03	2.24	2.47	90.2
casein + starch	200 °C, 1 h	8.52	2.33	3.01	89.1
casein + sucrose	200 °C, 1 h	7.94	3.15	3.61	86.5
casein + glucose	200 °C, 1 h	6.92	3.72	3.41	85.2
casein	300 °C, 1 h	7.28	1.28	1.25	47.8
casein + starch	300 °C, 1 h	4.43	0.00	0.19	4.3
casein + sucrose	300 °C, 1 h	3.75	0.21	0.03	6.3
casein + glucose	300 °C, 1 h	4.22	0.00	0.17	3.3

Ljungquist et al. (1979a,b) evaluated the plasma amino acid response in malnourished Ethiopian children fed wheat- and fish-based diets. Lysine and threonine were the limiting amino acids in the wheat diets but not in the combined wheat/fish diets. The authors concluded that (a) requirements for essential amino acids for young children are different from those for adults in terms of both absolute amounts and specific amino acids and (b) growth and simple maintenance have different amino acid requirements. Heat treatment caused significant losses in available lysine as determined by chemical analysis and rat bioassay. Plasma lysine levels in the human test subjects fed the heated milk showed corresponding lysine losses.

The utilization of food protein by ruminants is often enhanced by heat treatment, which can lessen protein breakdown by bacteria in the rumen (Friedman et al., 1982b). To optimize such heat treatments for soybeans, Faldet et al. (1992) measured available lysine *in vitro* and *in vivo*. Heat treatment that produced a loss of 15−22% of chemically available lysine also produced the optimal postruminal protection of proteins against bacterial degradation.

To fully understand the loss of lysine in foods, new and improved methods of lysine determination need to be developed. One such simple, rapid, and inexpensive method, we believe, is a modified ninhydrin assay for the measurement of available lysine which uses an improved lithium acetate−DMSO ninhydrin reagent (Friedman et al., 1984b; Pearce et al., 1988). This reagent (a) diffuses into complex food matrices, (b) rapidly extracts the proteins, (c) increases the rate of reaction between amino acids and ninhydrin, and (d) stabilizes the ninhydrin chromophore so that the solution can be stored in a refrigerator for up to 2 weeks.

Methionine. The low content of the essential amino acid L-methionine limits the nutritive value of many food proteins of plant origin, such as soybeans and other legumes. The problem is compounded by other factors. Methionine undergoes oxidation to methionine sulfoxides and methionine sulfone, racemization to D-methionine, and general degradation to compounds with undesirable odors and flavors such as methional. Protein-bound methionine from some plants is poorly utilized, presumably because of poor digestibility (Begbie and Fuszard, 1989).

We compared weight gains of mice fed amino acid methionine derivatives, isomeric dipeptides, and analogs (Friedman and Gumbmann, 1988). Since the mice received no other source of sulfur amino acids, the results reflect the ability of each compound to meet the animals' entire metabolic demand for dietary sulfur amino acids relative to L-methionine. Methionine peptides such as L-methionyl-L-methionine were not as toxic as L-methionine at high doses. The peptides may also alleviate the reported adverse flavor aspects associated with the addition of methionine to food.

Tryptophan. Tryptophan contributes to normal growth and protein synthesis and participates in numerous biochemical processes (Umezawa, 1989). Tryptophan is a second-limiting amino acid in maize. The stability of free or protein-bound tryptophan during processing and storage depends on temperature, radiation, and the presence of oxygen or other oxidizing agents such as lipid peroxides (Friedman and Cuq, 1988). In the absence of oxidizing agents, tryptophan is a stable amino acid, even in strongly basic or acidic conditions. At high temperatures and/or in the presence of carbonyl compounds, carboline is formed. Both carbolines and tryptophan-derived nitroso compounds are potential carcinogens. Tryptophan losses cannot always be monitored because of the lack of reliable analytical methods.

Cooked Foods. Sakamoto et al. (1992) demonstrated a direct relationship between the amino acid composition of 30 cooked Japanese foods and the net protein utilization (NPU) values for these same foods obtained from rat feeding studies. The best statistical correlation was based on ratios of lysine, sulfur amino acids, and tryptophan. They concluded that the ratio of essential amino acids in a protein rather than a single value for a limiting amino acid affects the nutritional value of a specific source of protein. The predicted net protein utilization (NPUp) based on amino acid analysis data does not require a digestibility factor. This *in vitro* technique merits further study to assess its potential to predict *in vivo* protein quality.

Other successful and unsuccessful attempts to predict the protein nutritional value of processed foods with the aid of regression equations based on amino acid composition are described by Happich et al. (1975), Martinez and Hopkins (1975), Marshall et al. (1979), Samman and Farias (1993), Schulz (1975), and Zarkadas (1992).

Liquid Diets. *Adult Formulas.* Zarkadas et al.

contents of several low-energy reducing diets. Calculated PER values based on amino acid analysis ranged from 2.5 to 2.7 for the casein and soybean diets and from 1.0 to 1.4 for the connective-tissue-based diets. The collagen contents of the connective tissue diets, determined from amounts of 5-hydroxylysine found in acid hydrolysates, ranged from 51 to 65%; total connective tissue proteins, determined from the amount of 4-hydroxyproline, ranged from 80 to 84%. The authors conclude that (a) evaluating the protein quality of low-energy diets can be based on amino acid composition and/or connective tissue protein content and (b) low-energy diets used without strict medical supervision can be life threatening.

Studies by Sarwar and Peace (1994) revealed that the protein quality of some liquid diets used in nursing homes is inferior to that of casein. The three limiting amino acids in these products were cysteine, threonine, and tryptophan. Kies (1989) measured the nutritional value of enteral formulas in humans, and Kozikowski and Zajka (1994) evaluated high-protein formulas containing hydrolyzed cheese fractions.

Lactation and Infant Formulas. Increasing the protein quality of a diet fed to pregnant rats also increased the milk yield 3-fold and the lysine content of mammary glands 6–8-fold, revealing a direct relationship between dietary lysine and free lysine in mammary glands, livers, and muscles: protein synthesis rate in the mammary glands; and milk yield. Increasing dietary protein quality or quantity also increased levels of most of the essential amino acids in the sera and brains of lactating rats (Jansen, 1988; Jansen et al., 1991). Evidently, the pattern of free amino acids in both brain and serum is strongly influenced by the amount and quality of protein consumed by rats during lactation. It is also noteworthy that the composition of mare's milk has a greater similarity to human than to cow's milk (Solaroli et al., 1993).

Hegazy et al. (1989) evaluated the nutritional potential of the following protein sources to serve as ingredients in infant feeding formulas: glandless cottonseed flour, skim milk, soybeans, rice, wheat, chickpeas, lentils, and semolina. The PER values of the various diets ranged from 1.83 to 2.85. They found that although the available lysine content was reduced during the extrusion processing of the various formulations, there was no correlation between the *in vitro* available lysine parameter and the *in vivo* PER values. Low-cost protein formulations for infant feeding may be as nutritious as higher-cost protein sources.

Hannaway et al. (1992) found that an experimental reduced-protein, milk-based formula with a whey/casein ratio of 40:60 fortified with tryptophan resulted in a tryptophan status in infants similar to that following breast feeding without compromising growth. Quinoa-based infant formulas are described by Najera (1992), and a hypoallergenic rice-based infant formula is described below in the section on rice. Related aspects are described by Asrephen and Wheat (1993), Grun et al. (1991), Rassin (1989), and Sarwar et al. (1993).

NUTRITIONAL VALUES OF DIFFERENT FOOD SOURCES

The earlier discussion shows that the nutritional value of protein depends on a number of variables including protein source, processing treatment, and method of assay used for its evaluation. With this as a background, I will now describe the nutritional values of a large number of protein sources subdivided into major categories. The specific source within each category is listed alphabetically.

Cereals. Acha (Digitaria exilis). According to de Lumen et al. (1993), acha, also known as fonio and "hungry rice", is a cereal that grows wild in Nigeria and other West African countries. Acha is high in methionine, making it a potentially good complement to legumes, which are low in methionine. The high methionine and cysteine contents (4.8 and 2.5%, respectively) of acha proteins could also serve as a means to detoxify the cyanide in cassava, widely eaten in Africa as a major source of carbohydrates.

Barley. Barley is the world's fourth most commonly used grain. Its major use is in brewing of beer. Natural populations of wild barley (*Hordeum spontaneum* = *H. vulgare* ssp. *spontaneum*) in Israel contain large amounts of untapped genetic material for improving the protein content of new varieties of barley. *H. spontaneum* grains have a protein content of approximately 21.5% (N × 5.7), 65% greater than that of Ruth, a standard Israeli barley cultivar, with 13% protein. A short-term nutritional study suggests that *H. spontaneum* would stimulate about a 50% greater weight gain in mice than would result from an equal weight of *H. vulgare*. *H. spontaneum* and other barley cultivars merit evaluation as a plant-breeding resource for the production of new barley varieties with high lysine and high protein content (Anjum et al., 1991; Friedman and Atsmon, 1988).

Buckwheat. The lysine content of Chinese buckwheat proteins is about twice that of wheat proteins (Wei et al., 1995).

Corn. Maize is the basic staple cereal grain for inhabitants of Latin America, Africa, and parts of Asia, where it provides more than half of the daily calorie and protein intakes. One of the most exciting discoveries in the area of cereal improvement was the discovery by Mertz (1978) that the opaque-2 gene in maize plants suppresses the synthesis of the nutritionally inadequate prolamine proteins, resulting in increased contents of lysine and tryptophan.

This section is largely based on the excellent overview by Bressani (1991) on the potential of high-lysine corn in human nutrition. Nitrogen balance studies with children show that the amount of nitrogen absorbed from opaque-2 corn-based diets (75%) approaches that of a milk-based diet (81%). The apparent digestibility in adult humans of the opaque-2 corn is similar to that of egg protein. Other nutritional benefits of the high-lysine maize compared to normal maize are (a) a higher food consumption, (b) a higher niacin availability, (c) a higher calcium utilization in tortillas processed with lime, (d) improved utilization of carbohydrates and carotene, and (e) nutritional security when corn is the sole source of protein. The nutritional superiority of the opaque-2 maize-based diets over normal corn diets is overwhelming. However, it remains to be shown whether high-lysine corn can be incorporated into foods acceptable to humans.

Finger Millet or Ragi (Eleusine caracana). Rao (1994) found that the PER of ragi, consumed by low socioeconomic groups in India, was lower than that of casein (2.5). The absence of tannins in the white variety may account for its higher PER (0.9) compared to the colored variety (0.7). Malting enhanced the PER of both varieties.

Grasses. Bargman et al. (1989) evaluated the composition and nutritional value of Eastern gamagrass (*Tripsacum dactyloidcs*), a warm-season perennial found predominantly in the eastern United States. Gam-

agrass, a relative of maize, has a high protein content but is poor in lysine. A nutritional evaluation using red flour beetle (*Tribolium castaneum*) larvae revealed that the nutritional value of gamagrass is also similar to that of maize.

Becker and Hanners (1991) feel that grains of perennial grasses such as wheatgrass (*Thinopyrum intermedium*) deserve development as alternative crops on marginal land. They found that Oahe Intermediate wheatgrass has a high protein content (20.8%), about double that of wheat. Although lysine is nutritionally limiting, wheatgrass contains higher levels of all the other essential amino acids than does wheat.

Rice. With a protein content between 5 and 7%, rice protein levels are lower than those found in most other cereals (Chang et al., 1986; Eggum et al., 1993; Gastanduy et al., 1990; Kennedy, 1975; Nishizawa et al., 1990; Sasslo, 1994). However, because the lysine content of rice proteins (3–4%) is more than 50% greater than that of wheat and because the amino acid balance is better, it is a better quality protein than wheat. Since rice constitutes a major food source for a large number of the world's population, extensive efforts have been made to discover new rice varieties with higher protein content without impairing the nutritional value. This is a challenging problem with a potentially large benefit-to-cost ratio. However, as appears to be the case with other cereals, most efforts to increase both the quantity and quality of rice proteins have been unsuccessful, apparently because there is generally a negative correlation between increased protein content and amino acid balance. It is hoped that this may not always be the case, as evidenced by some of the recent findings on the composition and nutritional value of the new varieties described below.

Nishizawa et al. (1990) describe the protein quality of several high-yield rice varieties and the consequences of lysine and threonine fortification. These varieties have very high protein (12%) and lysine (4%) contents and were also a good nutritional food for rats, with biological values (BV) ranging from 65 to 75% and net protein utilization (NPU) values from 55 to 65%. Fortification of the Milyang 30 rice variety with lysine plus threonine raised the BV from 70 to 85% and the NPU value from 60 to 75%. Plant breeding studies are needed to increase lysine and threonine content of high-protein rice to further enhance nutritional value.

Eggum et al. (1993) evaluated the digestibilities and nutritional values of cooked and milled rice in growing rats. They report that (a) the Filipino rice diets of both children and adults had a higher lysine content than milled rice; (b) milled rice is richer in cysteine and methionine; (c) amino acid digestibility of the rice diets ranged from 84 to 100%; (d) digestible energy was highest for the milled rice and lowest for the rice diets of preschool children, with adult rice diets in the middle; (e) the BV of the children's diet was 90%, of the adult diet, 86.6%, and of a cooked rice diet, 74.3%; (f) the corresponding NPU values were 80, 75, and 74%, respectively; and (g) the protein quality calculated from the amino acid score and true digestibility was 7–10% lower than actually observed in the animal feeding studies. The authors suggest that a better correlation between the *in vitro* and *in vivo* results is possible by using raw instead of processed (cooked and parboiled) rice in the digestibility assay.

Gastanduy et al. (1990) evaluated an infant formula of high protein rice flour (HPRF) fortified with lysine and threonine for malnourished male infants. They (80%); (b) N retention was equivalent to that of casein; and (c) because the rice-based infant formula was equivalent in nutritional value to that of the highest quality cow's-milk-derived formulas and because rice is a hypoallergenic food (HPRF), it can serve as a useful substitute for cow's milk in the feeding of children allergic to other foods.

Rice bran protein also appears to be nutritious (Prakash and Ramanatham, 1995). The calculated PER of a rice bran protein concentrate based on its amino acid composition (2.1) is similar to that of the observed PER based on animal studies (Bera and Mukherjee, 1988). Chang et al. (1986) describe the nutritional evaluation of high-protein rice, and Panigrahi et al. (1992) found that yellowing of white rice does not change its nutritional value.

Sorghum. Sorghum is a major cereal crop used in Africa and Asia as an animal feed and for making beer. McLean et al. (1981) evaluated the protein quality in children of 6–30 months of age of two high-lysine (2.9–3.0 g/100 g of protein) and two normal varieties (lysine = 2.1–2.2 g/100 g of protein) of whole grain sorghum milled as a flour. The mean absorption and retention of nitrogen were low with all diets. Essential amino acid levels in plasma were low, especially lysine and threonine. Lysine appeared to be the first limiting acid (Subramanian et al., 1990). The authors concluded that sorghum grain flour cannot be recommended for consumption by small children in view of the very poor energy and digestibilities observed.

Triticale. Triticale, a cross between rye and wheat, is being proposed as an alternative to wheat for commercial use (Heger and Eggum, 1991). Its yield, up to 1 ton of crude protein (CP)/ha, is generally superior to that of wheat. The biological value of the protein was higher than wheat (65.3 vs 61.6), presumably due to its higher lysine content (3.2 vs 3.0 g/16 g of N). These considerations have stimulated efforts to produce high-yielding triticale varieties. A breeding program in Czechoslovakia produced new triticale varieties with high contents of utilizable protein (UP).

The BV of triticale is 65%, and supplementation with lysine, methionine, threonine, and valine increases it to 94%, a value approaching that of lactalbumin (97%). The corresponding NPU values were 87 and 92, respectively (Heger, 1990).

Sikka et al. (1978) compared the growth of rats fed casein, triticale, and wheat. Growth rates with both cereal diets were significantly lower than with casein-based diets (Figure 1). They concluded that the superior growth rate with triticale compared to wheat is not due to differences in essential amino acids but to some other factor(s), possibly digestibility.

Wheat. Wheat protein is considered to be of poor quality, primarily because it has insufficient amounts of two essential amino acids: lysine, the first-limiting amino acid; and threonine, the second-limiting one (Arrage et al., 1992; Friedman and Finot, 1990; Leenars, 1993). Barabas and Barna (1989) examined PER values of a series of wheat proteins with high, intermediate, and low nitrogen contents. The nutritive value of these wheat varieties was inversely proportional to the protein content. Supplementation of the high-protein wheats with lysine produced growth approximating that obtained with casein. This was not the case for the wheats with a low protein content. An exception appears to be Durum wheat, the nutritional value of which in rats is better than that predicted on the basis of protein content. Breeding programs designed to optimize wheat varieties [illegible] on the basis of their

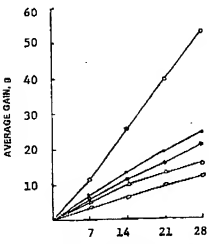

Figure 1. Weight gain of rats fed wheats, triticale, rye, and casein (Sikka et al., 1978).

nutritive value, due to lack of correlation between protein content and nutritive value and the fact that utilization of limiting amino acids varies.

The PERs of drum-dried wheat flakes (1.66) and unprocessed whole wheat (1.66) were greater than that of an extruded product (1.42) (Arrage et al., 1992).

The nutritive value of bread crust, fortified or not, was markedly less than that of crumb or whole bread. Lysine or glutamyllysine improved the protein quality (PER) of crumb more than that of either crust or whole bread, indicating a possible greater availability of the second-limiting amino acid, threonine, in crumb (Figure 2; Flodin, 1993; Friedman and Finot, 1990).

Legumes. *Castor Beans (Ricinus communis).* Putraj et al. (1994) found that boiling is preferable to lime/heat treatment to detoxify ricin present in castor beans.

Chickpeas (Cicer arietinum L.). Chickpea seeds, a member of the Leguminosae family, are an important food in southern Europe, the Middle East, North Africa, and India. In the United States, chickpeas are commonly used in salads. Chickpeas are low-fat legume seeds similar to fava beans, green peas, and lentils. Newman et al. (1987) found (a) similar PER values of about 2.8 for three chickpea varieties and (b) protein digestibilities ranging from 79 to 88%. These results show that chickpea protein quality is equivalent to that of soybean meal.

Del-Angel and Sotelo (1982) compared the nutritive value of mixtures of chickpeas with wheat, triticale, and normal and opaque-2 corn. The results showed that (a) although the protein contents of the normal and genetically improved opaque-2 corn were similar, the lysine, tryptophan, sulfur amino acid, and leucine contents of the opaque-2 corn and chickpea combinations were better balanced than those prepared with normal corn; (b) nutritionally optimum combinations consisted of triticale flour plus hard-endosperm opaque-2 corn; (c) the calculated scores based on amino acid composition correlated well with PER values based on rat feeding studies (r = 0.859); and (d) the use of genetically improved cereal–chickpea mixtures maintains high protein value and promotes desirable baking and other properties of cereals.

Combe et al. (1991) compared the utilization of amino acids from chickpeas, fava beans, and lentils. Methionine was not fully available to rats from any of the three legumes; threonine was not fully available from chickpeas; and arginine and lysine were not fully available from fava beans.

Common Beans (Phaseolus vulgaris L.). Dry beans are an important source of calories and proteins in Latin American countries and India. Dry bean proteins have a low nutritive value due to limiting amounts of sulfur amino acids, low digestibility, low bioavailability of essential amino acids, the presence of toxic and antinutritive factors, and the interference with digestion and absorption of nutrients by non-protein substances (Valenzuella and Sgarbieri, 1990).

To develop a better understanding of the influence of some of the cited factors that lower nutritional value, Valenzuella and Sgarbieri (1990) studied the nutritional consequences of feeding various dry bean fractions. They found that all bean fractions decreased rat growth, diet efficiency, and protein digestibility and utilization when added to casein diets. Although autoclaving of the

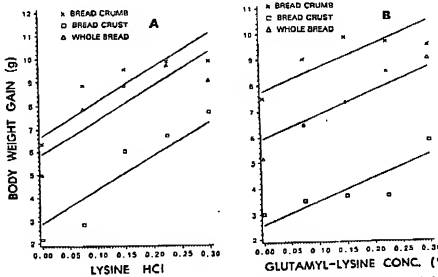

Figure 2. Weight gain in mice after 14 days of being fed whole bread, bread crumb, and bread crust cobaked with lysine (A) and

Table 5. Chemical Score (CS), Digestibility (D), Biological Value (BV), and Net Protein Utilization (NPU) of Selected Legumes (Burr, 1975)

food source	CS	D	BV	NPU
bean	54	73	58	42
broad bean	61	87	55	48
chickpea	63	86	68	58
cowpea	64	79	57	45
lentil	49	85	45	38
lima bean	64	78	66	51
pea	58	88	64	56
pigeon pea	42	78	57	44
peanut	65	87	54	47
soybean	74	90	73	66

fractions improved nutritional value in the mixed bean–casein diets, the values were still lower than with 10% casein alone. The observed differences in nutritional quality between the mixed dry bean–casein diets and the pure casein diets could not be explained by differences in amino acid scores. Evidently, not all heat-labile and heat-stable antinutritive factors present naturally in dry beans or those formed during processing have been identified and evaluated for their specific contribution to dry bean nutrition (Table 5).

Marquez and Lajolo (1990) found that dry beans appear to contain components producing undigestible protein complexes that influence amino acid utilization and endogenous nitrogen excretion. Surprisingly, supplementation of whole bean flour with albumin, which is rich in methionine, did not improve NPR. The expected benefit of supplementation was apparently masked by factors that depress nutritional value. These could include browning products generated by reactions of bean and albumin proteins with phenolic compounds and carbohydrates, interactions of fibers with intestinal mucosa, and unknown factors.

Cowpeas (Vigna unguiculata). Although cowpeas are the most important legume consumed in Africa, the plant has a low resistance to pests and plant disease. To circumvent this problem, extensive efforts are underway in Nigeria to develop pest-resistant varieties. To further this effort, Carnovale et al. (1991) compared the nutritional quality of wild and cultivated species of cowpeas. They found that (a) sulfur amino acids are limiting in both groups, (b) sulfur amino acids are not correlated with either protein or trypsin inhibitor levels, and (c) selected lines improve nutritional quality due to lower inhibitor and tannin contents. These considerations suggest that the search for high-quality pest-resistant cowpea varieties needs to be continued.

Nell et al. (1992) found that the average protein content of 150 different cowpea cultivars was 28.4 ± 1.8%. Although autoclaving generally improved nutritional value, the true digestibility value of autoclaved cowpea meal (76) was lower than that of lactalbumin (92). The RNV of cowpeas was 0.59 compared to 1.0 for lactalbumin. The authors concluded that (a) cowpeas are a valuable protein source that can overcome predicted protein shortages; (b) protein contents had no effect on protein quality of the meals evaluated, suggesting that selection of better cultivars can be based on protein content alone; and (c) additional studies are needed to find the best treatment to remove nonnutritional lectins, protease inhibitors, and other factors to lower cost and acceptability of the final processed meal (Tuan and Phillips, 1992).

Desert Ironwood (Olnea tesota). Desert ironwood is a leguminous tree common in North American deserts. Feeding seed meal to rats resulted in an adjusted PER [illegible] of 91% (Becker 1983). The tree contains the unusual amino acid canavanine, which is not extracted by cooking methods.

Fava Beans (Vicia fava). Fava beans, also known as faba beans, are a widely used protein source (Pastuszewska et al., 1993; Rubio et al., 1991). Some people with an inborn deficiency of glucose-6-phosphate dehydrogenase (G6PD) suffer from a hemolytic crisis known as favism when they consume fava beans. Studies of the pathogenesis of this disease suggest that isouramil (2,4,6-trihydroxy-5-aminopyrimidine) and divicine (2,6-diamino-4,5-dihydroxypyrimidine), the aglycons of the β-glycosides convicine and vicine, respectively, appear to be responsible for the hemolysis (Liener, 1975). Elsewhere, I describe a possible mechanism for the pathogenesis of favism involving reduced glutathione (Friedman, 1973).

Lentils (Lens culinaris Medkus). According to Savage (1991), lentils were among the earliest crops cultivated by Neolithic man. The bean is now an important dietary source for humans. Since legumes such as lentils now provide about 10% of the world's total dietary protein and have 2–4 times the protein content of cereals, their use as a human food is expected to grow. The high saponin content of lentils (3.7–4.6 g/kg) may have health benefits by reducing cholesterol. Antinutritional and potentially toxic factors in lentils include (a) carbohydrates such as raffinose, which may induce flatus; (b) trypsin inhibitors, which are largely destroyed by boiling at 100 °C or by germination of the seeds; and (c) lectins, which are growth depressing at low levels in the diet and toxic at high levels. Nevertheless, increased consumption of indigenous legumes such as lentils and soy beans may be a practical means to reduce human malnutrition, provided suitable strategies are devised to inactivate or remove the antinutritional and toxic factors (Liener, 1994).

Lupin (Lupinus polyphyllus). Lupins grow well in cool climates and on slightly acidic soils. Lupin seeds have a high protein content and nutritive value, and, unlike soybeans, do not contain antinutritional factors such as lectins and protease inhibitors (Aniszewski, 1993). They could serve as an excellent food for humans. Unfortunately, they also contain a large number of quinolizidine alkaloids, which make the seeds bitter and potentially toxic. At present, these compounds must be removed by cost-increasing postharvest processing. Extensive efforts are underway in many parts of the world to develop low-alkaloid lupins.

Donovan et al. (1991) report that the nutritional quality of two lupin (*Lupinus albus*) cultivars ("sweet white lupin") with a low quinolizidine alkaloid content (0.05%) supplemented with 0.2% L-methionine was similar to that of soybean meal. The NPR values for both lupins was about 80%, less than that of casein (100%). Aniszewski (1993) describes a comprehensive compositional and nutritional study of a new lupin variety. He reports that (a) the alkaloid content of the new variety is low; (b) the seeds contained 16 different alkaloids but did not contain the highly toxic alkaloid anagyrine, widely found in other varieties; (c) the seed protein content was 37.7%; (d) the proteins are rich in lysine and poor in methionine; and (e) although an improvement over earlier genotypes, these seeds are probably still not useful as a human protein source. Further selection is needed to accomplish this objective.

Mesquite Pods (Prosopis spp.). According to Becker et al. (1992), pods of mesquite, a leguminous tree, have traditionally been used as a source of animal and human food in desert cultures, especially by Native Americans. Although the protein of raw pods is deficient in sulfur

amino acids, the tree merits further development as a source of forage and fodder.

Mung Beans (Vigna radiata). According to Savage and Deo (1989), *V. radiata* beans, which include mung beans and Urd, are used as a major supplementary protein source in many countries. They contain low levels of fat and fiber. The PER values of the bean is 1.6, increasing to about 2.1 after roasting, still much less than that of casein. However, supplementation of the bean with methionine and reduction of antinutritional factors including lectins and protease inhibitors increase this value considerably.

Nuña Popping Bean. Nuñas (*Phaseolus vulgaris* L., Fabaceae) are cultivated in the Andean mountains of Ecuador and Bolivia. Van Beem et al. (1992) report that the beans are widely used as a snack after toasting. The 3 min toasting treatment partly reduces both their lectin content and digestibility while not affecting tannin levels.

Peanuts (Arachis hypogaea). Chuman et al. (1990) measured the protein quality of peanuts using both *in vitro* and microbiological *in vivo* assays. They found that the chemical score for lysine and sulfur amino acids ranged from 37 to 50% and from 47 to 55%, respectively, for eight peanut cultivars tested. PER and RNV for the same samples ranged from 1.45 to 1.76% and from 45.6 to 54.2%, respectively. PER was significantly correlated with chemical score ($r = 0.88$) as was RNV ($r = 0.98$). The results show that the protein quality of peanuts is not very high. Hazelnut protein complements the nutritional value of pea protein (Villaroel et al., 1990)

Pigeon Pea (Cajanus cajan L.). Pigeon pea, a tropical bush legume also known as red gram, is widely used as a food source in Africa and India. Griffiths and Savage (1991) reviewed the composition and nutritive value of pigeon pea. They reported that (a) the composition of the pea does not differ greatly from that of other legumes; (b) the proteins are deficient in methionine and tryptophan; and (c) the peas contain several antinutritive factors such as lectins, protease inhibitors, amylase inhibitors, cyanogenic glycosides, oxalates, phytates, and tannins. Positive nutritive factors in the peas lower cholesterol and serum glucose and quicken adjustment to altitude stress. The latter may be due to the ability of the peas to protect against altitude-induced increases in serum arginase and serum ornithine decarboxylase activities. These considerations suggest the need to create protein-rich and better amino acid-balanced pigeon pea varieties.

Singh et al. (1993) report on active research efforts to develop high-protein genotypes with improved nutritional quality. Several such genotypes are now available, with a protein content of up to 24 g/100 g of dehulled seeds. These legume seeds are high in lysine (6.7 g/100 g of protein), but their combined cysteine and methionine content of 2.5 g/100 g of protein is no different from that of standard genotypes. In addition to sulfur amino acids, tryptophan was also nutritionally limiting in these seeds. The seeds are well digested and have a biological value of about 66%, a net protein utilization value of about 60%, and about 13% utilizable protein. They are also a good source of calcium. The new seeds contain more calcium than standard varieties.

Rice Bean (Vigna umbellata). Rice bean is a legume indigenous to the Philippines, India, and China. Because the plant resists pests and disease, it could provide a useful source of protein for both animals and humans. Rodriguez and Mendoza (1991) found that *in*

Table 6. Protein Efficiency Ratios (PER), Inhibitor Content (Milligrams per Gram), and Pancreas Weight Ratios of Rats Fed Heated and Untreated Soy Flours (Friedman et al., 1991)

diet	PER	trypsin inhibited	chymotrypsin inhibited	pancreas wt relative to body wt
Williams 82 flour				
unheated	−0.14	36	4.2	0.806
heated for 10 min	1.42	25	0.8	0.572
heated for 20 min	2.13	7	0	0.427
heated for 30 min	2.22	6	0	0.446
L81-4590 flour				
unheated	0.46	20	3.3	0.721
heated for 10 min	1.63	12	0.7	0.503
heated for 20 min	2.25	0.8	0	0.430
heated for 30 min	2.28	0.6	0	0.431
casein control	3.27			0.466

to 86%. The low nutritive value of mature seeds measured as RNV in rats reached 79% after boiling or roasting. Sulfur amino acids were the most limiting in the rice bean diets.

Soybeans. The composition and nutrition of soybeans have been studied extensively (Friedman et al., 1984a, 1991; Fukushima, 1991; Gumbmann and Friedman, 1987; Istfan et al., 1983a,b). According to Fukushima (1991), the following soybean food products are widely used in Eastern Asia: soy sauce, *miso* (fermented soybean paste), *natto* (fermented whole soybeans), *tempeh* (fermented and deep-fat-fried whole soybeans), *sufu* (fermented soybean protein curd), *kinako* (roasted soybean flour), *tofu* (soybean protein curd), *aburu-age* (deep-fat-fried soybean protein curd), soy milk, and *yuba* (soybean protein film made from soy milk).

Istfan et al. (1983a) compared the nutritional value of a soy protein concentrate with a milk protein in young adult men using a 10 day nitrogen balance method. They found that soy protein supported nitrogen equilibrium as well as the animal protein. The mean daily intake of soy concentrate sufficient for nitrogen balance was 95 mg of N/kg. These studies imply that since soy protein is nutritionally equivalent to animal proteins such as egg, milk, fish, and beef, foods containing soy protein merit wider use in human nutrition. Istfan et al. (1983b) also demonstrated that a well-processed soy concentrate can serve as a sole source of nitrogen and essential amino acids for long-term maintenance in adult humans.

Amino acid patterns by themselves may be insufficient to predict utilization of a protein. In soybeans and other legumes, antinutritional factors such as inhibitors of digestive enzymes and hemagglutinins, as well as poor digestibility are all reported to lower nutritional value. Table 6 shows that raw Williams 82 soy flour produced a negative PER (weight loss) (Friedman et al., 1991; Liener, 1994; Liener et al., 1988). Heat improved the nutritional quality of the product.

Adverse effects following short- and long-term ingestion of raw soybean meal by mammals and birds have been attributed to the presence of soybean protease inhibitors and lectins. To minimize possible human health hazards and to improve the nutritional quality of soy foods, inhibitors are generally inactivated by heat treatment during food processing or are removed by fractionation. Most commercially heated flours retain 5–20% of the original trypsin and chymotrypsin inhibitor activity. The more protracted heating required to destroy all inhibitor activity would damage the nutritive value of soy proteins. Friedman and colleagues successfully developed improved ways to inactivate soybean inhibitors through disulfide interchange. The beneficial

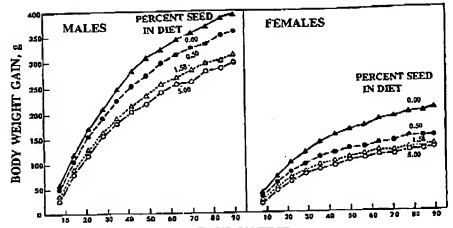

Figure 3. Body weight gain in rats fed jimson weed for 90 days (Dugan et al., 1989).

nutritional consequences are described elsewhere (Table 6; Friedman and Gumbmann, 1986a,b; Friedman et al., 1984a).

Screening of several accessions from the USDA Soybean Germplasm Collection showed variation in the content of trypsin inhibitor, sulfur amino acids, and lectins, indicating that further screening studies could lead to the discovery of soybeans which yield flour that is safe and nutritious with minimal heating (Domagalski et al., 1992).

Tamarind (Tamarindus indica). Bhattacharya et al. (1994) isolated a protein rich in lysine but poor in sulfur amino acids from the kernels of the tamarind legume tree. It had an *in vitro* digestibility of 71.3. The seeds are a byproduct of the tamarind pulp industry.

Other Seeds. *Cottonseeds.* Martinez and Hopkins (1975) evaluated protein quality of several cottonseed protein products. They report that the quality of defatted cottonseed depends on the level of active or "free" gossypol present and the degree of damage incurred by the protein during defatting. Since gossypol in the seed is potentially toxic to monogastric animals and its aldehyde group can also interact with the amino groups of lysine, damaging protein quality, it should be removed before processing of the seeds. These authors also found that defatted cottonseed flour processed to remove gossypol has a PER value near that of casein.

Anderson et al. (1984) measured lysine damage in protein treated with gossypol. They found that the reduction of *in vivo* digestibility of cottonseed flour, egg white, and ovalbumin due to gossypol treatment explained the differences observed between the values for chemically reactive lysine measured *in vitro* from rat growth assays (Feggeros et al., 1992).

Muskmelon (Cucumis-melo L.). Karakaya et al. (1994) obtained an index of nutritional quality of the protein present in melonseeds. A value of 1.0 indicates that a food supplies the nutrient need in the same proportion as the caloric need (Hansen, 1973).

Pumpkin Seeds (Curcurbita pepo). Mansour et al. (1993a) reported that pumpkin seed is cultivated commercially in Hungary. They found that pumpkin (Kakai 35) seeds' protein content is high (32 g/100 g of seeds), the amino acid pattern of the seed protein is similar to that of soybeans except for a lower lysine content, and both *in vitro* digestibility and biological value of the seed proteins are high. Pumpkin seed products are a good

Rapeseeds. Larbier et al. (1991) compared the nutritional value in poultry of whole and dehulled rapeseed meal to that of soybean meal. The available lysine contents of the three meals determined in the growth assays were 72.8, 78.3, and 87.5%, respectively. The corresponding true digestibilities were 78.9, 81.4, and 87.5%, respectively. There is evidently a close relationship between these two indices of protein quality for the three protein sources tested. Rapeseeds with a low glucosinolate content are a potential major source of edible protein (Mansour et al., 1993b).

Sunflower Seeds (Helianthus annuus L.). Mastrodi Salgado and Chieus (1988a,b) evaluated the nutritional quality of sunflower seeds, which are widely used as a source of oil. The low PER of sunflower seed flour (1.5) was improved when 0.34% lysine was added but did not change with a corresponding supplementation with methionine. Complementation of sunflower protein concentrate with sesame flour, rich in methionine, also did not change PER. In contrast, complementation with fish meal, rich in lysine, raised the PER to 2.2. Lysine is nutritionally limiting in sunflower seed proteins. The high protein content of the seeds (> 50%) suggests that they can provide a good source of both oil and protein (Vieira et al., 1992).

Weeds. Weeds are widely distributed among most harvested plant material. They often enter into and contaminate the food chain. For example, commercial grain may contain jimson weed (*Datura stramonium*) seeds. The tropane alkaloids in these seeds, atropine and scopolamine, are potentially toxic. To evaluate the nutritional and toxicological potential of jimson weed seeds, diets containing 0.5, 1.58, and 5.0% of these seeds were fed to male and female rats in a 90 day subchronic feeding study (Dugan et al., 1989; Figure 3). The main effects were decreases in body weight gain, serum albumin, and serum calcium and increases in liver and testes weight gains, serum alkaline phosphatase, and blood urea nitrogen. Jimson weed seeds at a concentration of 0.5% or more in the diet produced adverse physiological changes in the rat, presumably due to the presence of the alkaloids since the protein contents of the seeds is intermediate between that in cereals and that in legumes.

Fokou and Domnangang (1989) state that a need exists to develop new protein sources because many people cannot afford expensive meat proteins or will not eat meat for ethical and religious reasons. To help meet

Table 7. Amino Acid Content of Defatted Soy and Weed Seed Flours (Milligrams per Gram of Protein) (Crawford et al., 1980; Friedman and Dao, 1992; Friedman and Levin, 1989)

amino acid	soy flour	wheat flour	jimson weed	morning glory	sickle-pod	velvet-leaf
Asp	117	41	77	138	111	106
Thr	36	25	31	48	44	35
Ser	49	43	40	62	56	49
Glu	186	331	130	207	158	174
Pro	52	115	33	50	40	
Gly	40	35	39	65	51	97
Ala	40	28	35	65	48	83
Cys	52	41	36	60	55	20
Met	11	22	20	21	18	16
Val	12	16	14	15	17	63
Ile	47	35	32	31	44	40
Leu	77	67	53	97	81	80
Tyr	34	32	25	33	33	26
Phe	50	48	35	57	48	41
His	25	21	18	34	27	24
Lys	58	18	32	71	70	54
Arg	73	36	65	80	76	32
Trp	11	5	5	6	16	

these needs, these authors evaluated the nutritional value in rats of proteins in the leaves of *Solanum nigrum* and other related leafy green vegetables that are widely consumed in Africa. They found PER values of 2.08–2.18 and ascribe the relatively poor quality of these leaves to the presence of saponins and alkaloids, which in rats are known to decrease food consumption and to be potentially toxic.

If it were possible to reduce or eliminate the alkaloids or other toxic compounds by breeding or molecular biological techniques, such wild vegetables and weeds could provide useful new sources of proteins and other nutrients (Table 7; Sotelo et al., 1993).

Animal Protein. *Meat.* Meat is widely used as a nutritional source of protein (Hoffmann, 1993). The major meat proteins include actin, collagen, and myosin. Commercial meat products vary widely in their contents of connective tissue, myofibrillar, and nonmuscle proteins. Meat proteins contain amino acids not usually found in plant proteins, such as methylhistidine and hydroxymethyllysine.

Zarkadas (1992) measured the amino acid profiles of several commercial meat products and calculated predicted PER values. He states that (a) the amino acid data are best expressed as grams of anhydrous amino acid per kilogram of total protein; (b) the 4-hydroxyproline content of composite meat products varies widely. (c) the composite meat products evaluated contain significant amounts of essential amino acids; and (d) calculated PER values of various meat products based on amino acid data ranged from about 2.7 to 3.2, values above the 2.5 required for meat in the United States (FSIS, 1984). Predicted PER based on collagen content for meat samples in the range 2.22–2.91 agree with rat PER values (El, 1995).

Seafood. Seafood, including fish, is a major source of dietary protein. The PER values of most fish range from 3.1 to 3.7 (Sidwell and Ambrose, 1975). The nutritional values of most fish proteins are equal to or better than that of casein. Evidently, the protein quality of most fish may exceed that of meat and be equal to that of an ideal protein such as lactalbumin.

Machado and Sgarbieri (1991) found that the PER of proteins from pacu (*Colossoma mitera*), a freshwater fish from Brazil, was similar to that of casein. However, the biological value was lower than found with casein

Nakajima et al. (1990) examined the amino acid composition and nutritive quality of pearl oyster proteins. Growth rates of rats fed these proteins were better than those fed casein. Amino acid scores of a series of shellfish ranged from 68 for abalone to 95 for carb shell, with widely consumed scallops (about 71) and clams (up to 87) having intermediate values. Pearl oysters also significantly decreased plasma cholesterol, which went from 82 mg/dL with the casein diets down to 57 mg/dL with the oyster-fed rats. Shellfish provide a high-quality protein, with the first limiting amino acids being either leucine or valine.

Other Sources. *Amaranth (Amaranthus cruentus).* Betschart (1982) suggests that amaranth grain has high potential as a food/feed crop because it grows rapidly in regions where temperate crops do not. They report that hot-air popping resulted in a PER value of 1.7 and an apparent digestibility of 77%, values similar to those obtained with control seeds. Amaranth has a relatively high lysine content (about 5 g/16 g of N) with leucine being the first limiting amino acid. *A. cruentus* compares favorably with many other protein sources in terms of protein quality and quantity (Acar et al., 1988).

De Mucciarelli et al. (1990) evaluated the nutritional quality of amaranth flour. They found that (a) the flour had a protein content of 21.7% and an available lysine content of 5.2 g/16 g of N, (b) the calcium content (500 mg/100 g) was remarkably high, and (c) the quality of the protein (NPR = 2.1) was acceptable. These results show that amaranth seeds are high in both protein and lysine and suggest that it merits wider use in cereal-based diets.

Breadfruit. Breadfruit is rich in carbohydrates, but its protein content of 4% is about one-third that of wheat. Nochera and Caldwell (1991) found that acceptable breads can be formulated containing about 10% breadfruit flour. Because wheat production in tropical countries is limited, readily available breadfruit could supplement part of the wheat used for bread-baking. These authors reported that breadfruit-containing biscuits had a PER value of 2.2, approaching that of casein (2.5). In contrast, the composite bread's PER of 0.87 was similar to that of all-wheat bread. It is not immediately apparent why the two baked products differ so greatly in PER.

Carrots. The protein quality (BV = 77–82) and energy density of carrots are influenced by variety and N content of the soil (Bruunsgaard et al., 1994a).

Cassava (Manihot esculenta Crantz). Cassava or cassava is widely used as a high-energy food in Africa. The protein content of cassava is low (4% of dry weight). Methionine detoxifies the cyanogenic glycosides linamarin and lotaustralin present in cassawa, which produce HCN. Brough (1992) reviewed extensive efforts to prepare safe cassawa food products. He stated that supplementation of cassawa with methionine significantly improves growth, feed conversion efficiency, and cyanide detoxification. A challenging problem is to develop cassawa plants that are low in cyanogenic glycosides.

Crisphead Lettuce. The protein value (BV = 77–90) and energy density of crisphead lettuce are influenced by the N content of the soil and depend on plant variety (Bruunsgaard et al., 1994b).

Insects. Finke et al. (1989) demonstrated the potential value of insects as sources of high-quality protein.

Jojoba Shrub (Simmondsia chinensis). Nuts of the jojoba shrub produce a valuable oil. Studies by Booth et al. (1974) and Cokelaere et al. (1993) demonstrated that feeding the protein-rich oil-extracted jojoba meal

to rats depressed growth, presumably because of the presence of the toxic glycoalkaloid simmondsin. If simmondsin and related glycoalkaloids could be removed during food processing or their biosynthesis suppressed by plant molecular biology techniques, the jojoba shrub could provide both oil and edible protein.

Leaf Protein. The most abundant source of protein for most animals is leaves. The potential benefit of leaf protein for human nutrition is enormous. However, the use of leaves as a protein source for human consumption has been limited due to nondigestible fiber content, the presence of chlorophyll and phenolic compounds that affect nutritional properties, and unattractive organoleptic properties such as grassy flavors and odors. Extensive efforts have been made to overcome these problems by concentrating leaf protein. Several recent studies that show the continuing interest in this area of prorein nutrition are briefly summarized here.

Hanczakowski et al. (1991) found that fortification of the leaf protein from lucerne (*Medicago sativa*) with methionine increased the biological value (BV) of the protein from 41 to 68 in rats, suggesting that methionine is the first limiting amino acid in leaf proteins. In contrast, supplementation with lysine had no effect.

De Stefanis et al. (1990) reported that fortification of pasta with a protein concentrate isolated from tobacco significantly improved nutritional quality. However, this was not the case with a concentrate isolated from Jerusalem artichoke (*Helianthus tuberosis*). The authors suggested that addition of leaf protein to pastas and other foods merits further exploration.

Herrera and Rosas Romero (1990) found that the presence of sodium sulfite during the alkaline extraction of leaf protein from plantain (*Musa paradisiaca* L.) at 85 °C resulted in a better quality protein than did extraction without sulfite. The protein quality was further increased to a PER of 1.9 by supplementation with methionine.

Hanczakowski and Szymczyk (1992) suggested that because isolation of proteins from leaves often requires alkaline conditions and heat, which can damage protein quality, it may be preferable to squeeze protein-containing juices from the leaves. They report that the protein quality of juices derived from grasses did not differ significantly from that obtained from barley leaves. Evidently, the quality of the protein in many leaves is similar.

Yeoh and Wong (1993) showed that leaves of several tropical vegetable plants grown in Malaysia and China are protein-rich, with protein contents ranging from 3 to 4.6% of fresh weight. They have a good complement of essential amino acids and do not contain protease inhibitors or cyanide. These plants are potential food sources that merit wider use.

Mushrooms. The amino acid composition of mushrooms suggests that they could serve as a source of high-quality protein (Danell and Eaker, 1992; Eder and Wunsch, 1991; Gupta and Singh, 1991; Kurtzman, 1975). This conclusion was confirmed by Eder and Wunsch (1991), who evaluated the protein quality of oyster mushrooms on the basis of amino acid composition data. They found that about 40% of the total nitrogen content of dry mushrooms was contributed by protein amino acids. The essential amino acid index calculated from the amino acid composition was above 85, showing that mushroom protein quality may approach that of animal protein. The digestibility of mushroom crude protein is about 78%, compared to 100% for an ideal protein. These considerations suggest proteins. However, some caution may be in order because chitin and other non-protein nitrogen compounds may interfere with the determination of total protein contents of mushrooms.

The high lysine and sulfur amino acid content of truffles suggests that their protein content is of a high-quality (Colì et al., 1988).

Potatoes (Solanum tuberosum). Potatoes are commonly perceived as a carbohydrate source only, but they are also a good source of high-quality protein. Although potatoes contain only about 2% protein on a fresh basis, the value increases to about 10% when examined on a dry basis, equal to that of most cereals such as rice or wheat (McKay et al., 1987).

A summary of the nutritive value of potato protein by Markakis (1975) shows the following: (a) Only about 50% of the total nitrogen of potatoes is derived from proteins; the remaining nitrogen consists of free amino acids (15%), amide nitrogen associated with asparagine and glutamine (23%), and non-protein nitrogen associated with the glycoalkaloids solanine and chaconine and secondary metabolites such as acetylcholine, adenine, cadaverine, guanine, hypoxanthine, narcotine, trigonelline, and xanthine (12%). (b) On the basis of amino acid composition, the calculated protein quality is about 70% that of whole egg protein. (c) Potatoes provide an excellent source of lysine, but low contents of sulfur amino acids limit their nutritive value. (d) Human feeding trials suggest that potato proteins are of a very high quality, possibly higher than indicated by the amino acid composition. This may be because protein utilization is enhanced by the high content of free amino acids and other metabolites mentioned earlier.

In a recent study, Eppendorfer and Eggum (1994) found that a large amount of nitrogen fertilizer increased the quantity and reduced the quality of potato protein.

Nestares et al. (1993) found that the potato concentrate's nutritional quality was excellent when measured in terms of protein efficiency ratio (PER = 2.90), biological value (BV), net protein utilization (NPU), and nitrogen retention. These results reinforce the idea that potato protein merits inclusion in various food formulations as a source of high-quality protein.

Kies and Fox (1972) fed human volunteers potato protein (derived from dehydrated flakes) with and without supplementation. They reported that (a) the mean crude protein digestibility of the potato protein was 78% and (b) the mean nitrogen balance of the human subjects increased when the potato protein was fortified with 0.3% methionine, but not with leucine or phenylalanine. These results suggest that complementary diets consisting of potatoes, which are high in lysine but low in sulfur amino acids, and cereals, which are low in lysine but high in sulfur amino acids, should provide a well-balanced protein source. They also imply that a need exists to develop new potato cultivars high in both protein and sulfur amino acids.

Since potatoes need to be baked, boiled, fried, or otherwise cooked before consumption, it is of interest to find to what extent such exposure to heat affects nutritive value. According to Jaswal (1973), losses during canning, chipping, and drum-drying are considerable but appear to be minor during boiling and frying (Friedman, 1992a,b).

Quinoa (Chenopodium quinoa Willd.). Quinoa, a pseudocereal indigenous to Latin America, constituted a significant part of the diet of the pre-Columbian Andean Incas (Kozio, 1992). Because of its high nutritional quality, quinoa was able to supplement and

often replace animal protein in the diet. The plant grows well in cool climates and at high altitudes. Quinoa is called "mijo" or little rice in Spanish since its seeds resemble those of rice plants.

Quinoa is a good source of high-quality protein. Compared to cereals, its seeds contain more histidine, cystine, methionine, and isoleucine. The seeds are especially rich in lysine, which comprises about 6% of the protein. The well-balanced nature of quinoa proteins (about 15% of the fresh weight of the seeds) suggests that nutritionally they may be as good as milk proteins. The PER of cooked quinoa exceeds that of casein and is superior to that of wheat.

Quinoa also contains several antinutritional compounds such as phytic acid, tannins, and trypsin inhibitor, which could affect its nutritional quality. The effects of these can be minimized by postharvest processing or through selective breeding of improved varieties. If the cost of quinoa seeds could be lowered, they could find uses in infant formulas and in improving the nutritional values of baked and cooked cereal products such as bread, breakfast cereals, and pasta (Najera, 1992; Ranhotra et al., 1993).

Raspberry Pomace. Raspberry pomace is the residue remaining after raspberries have been processed for juice production. Although the pomace contains only 1.5% digestible protein, its biological value ranged up to 91% (McDougall and Beames, 1994).

Seaweeds. Akhilender et al. (1993) evaluated the nutritional quality and safety of Indian seaweeds. The seaweeds had a lower PER value than that of casein. Acute and subacute oral feeding of the seaweeds for up to 12 weeks did not produce any toxic effects in rats.

Tomatoes (Lycopersicum esculentus). Udayasekhara (1991) measured the nutritional value of defatted tomatoes. The cake was rich in protein (40%) and minerals. The lysine content (5.1%) was comparable to that found in groundnut protein. The protein quality of the tomato cake was lower than that derived from groundnuts, presumably because sulfur amino acids are nutritionally limiting in tomatoes but not in groundnuts. The PER values of tomato processing byproducts were low (1.54–1.64) compared to that of casein (2.5) (Barcelos et al., 1992). Tomato seeds were not toxic when fed up to 4% in a casein-based diet (Friedman, 1992b).

Yeast Proteins. Kinsella and Shetty (1978) described procedures to reduce the ribonucleic acid (RNA) content of yeast proteins to make them edible. They suggest that microbial proteins derived from yeast and other sources could contribute to the solution of the protein–calorie malnutrition problem.

RESEARCH NEEDS

The preceding analysis of the current status of protein nutrition shows that this is an evolving area of food science with many unsolved problems. To facilitate progress, the following sections describe additional important factors that impact protein nutrition and recommend research important to human well-being.

Protein Quality Assessment. A 14 day mouse feeding study to measure protein quality merits further evaluation for practical use as possible replacement of the PER method because it takes less time and is estimated to cost about one-fifth as much as the standard PER method (Friedman, 1992a; Gorski et al., 1991; Keith and Bell, 1988). The mouse assay may also be competitive with the method based on an amino acid score corrected for digestibility that has been recommended [illegible] the PER, as discussed earlier. The food industry should also consider using the net protein ratio (NPR) method, which requires 14 days and corrects for endogenous losses of protein. Because of differing requirements for sulfur amino acids, nutritional values obtained with rodents may not apply to humans. Rodent data can be used to define relative quality of different protein sources.

Protein Quality Improvement. *Mixed Proteins.* In many foods, especially those of plant origin, low levels of various essential amino acids limit nutritive value. This is particularly important for cereals, which may be inadequate in the essential amino acids isoleucine, lysine, threonine, and tryptophan, and legumes, which are a poor source of methionine. Since these commodities are the principal source of protein for much of the Earth's population, a need exists to overcome this problem. These inadequacies in nutritional value of major protein sources can be solved in at least three ways: (a) combining protein sources to create mixtures with an adequate amino acid balance; (b) fortification of the low-quality proteins with essential amino acids; and (c) developing high-quality plant proteins by breeding or molecular biology techniques. The following brief summary focuses mainly on the first approach.

Woodham (1978) analyzed theoretical and practical aspects of evaluating the nutritive value of mixed proteins. He concluded that (a) mixtures of proteins fed to animals often result in better growth than would be expected when each component of the mixture is fed separately; (b) the improved growth can be attributed to the improved amino acid balance of the mixed diet; (c) amino acid excesses of mixed diets may actually be deleterious; and (d) diet balancing should be designed to both minimize deficiencies and reduce excesses of amino acids.

This latter problem is elegantly discussed by Benevenga and Cieslak (1978), who stated that (a) the slope-ratio technique widely used to measure protein quality of mixed diets may not be linear with protein concentration in the diet; (b) animals can adapt and make more efficient use of diets low in protein quality and/or quantity; and (c) protein mixtures should be evaluated under specific conditions of potential use, e.g., maintenance, normal growth, or maximum growth, which may require different ratios of the proteins for optimum results.

Practical problems associated with measuring protein nutritive quality of mixtures are illustrated by a study designed to optimize nutritional values of mixtures of dry-roasted navy bean flour with various cereal grains such as barley, buckwheat, corn, rice, and wheat germ (Yadav and Liener, 1978). Figure 4 illustrates complementary effects and the roles of lysine and sulfur amino acids in roasted navy bean–corn mixtures in defining PER. Table 8 lists amino acid scores of some mixed protein sources with and without amino acid fortification. The following additional studies demonstrate the nutritional benefits of complementary food sources. Abrahmson et al. (1974) demonstrated a strong correlation ($r = 0.96$) between PER and chemical scores in several mixed diets. Abreu et al. (1994) showed that supplementation of wheat flour with amaranth improved protein quality of the flour by about 45%. Asledu et al. (1994) found that the nutritional value of corn flour was significantly improved by the addition of fish protein. Dodok et al. (1993) found that bread and biscuits containing 10–20% chickpea flour had a higher nutritional value than the baked goods made from wheat flour alone. Barron and Espinoza (1993) reported that tortillas fortified with chickpeas had a significantly

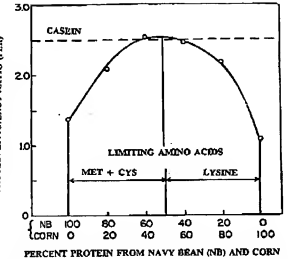

Figure 4. Complementary nutritional effects produced by mixtures of navy beans (NB) and corn at a protein level of 8.3% [adapted from Yadav and Liener (1978)].

Table 8. Amino Acid Scores (AAS) of Selected Food Proteins with and without Fortification by Essential Amino Acids (Sarwar et al., 1978)

protein source	most limiting amino acid	AAS, % mean
wheat flour	Lys	49
wheat flour + egg white (60:40 protein)	Lys	80
wheat flour + soy protein (60:40 protein)	Lys	76
wheat flour + rapeseed concentrate (50:50 protein)	Lys	77
casein	Met + cystine	99
minced beef	Met + cystine	93
pea flour	Met + cystine	74
soy protein	Met + cystine	69
wheat flour + L-lysine (200 mg/g of N)	Thr	73
soy protein + L-methionine (100 mg/g of N)	Thr	96

higher nutritional value than unfortified corn tortillas. Naikare and Mabesa (1993) showed that infant formula containing a mixture of rice, mungo, sesame, and carrots had a PER of 2.4. Joseph and Swanson (1993) found that the protein quality of beans was improved by combining them with rice. Finally, Marquez et al. (1992) discovered that the NPR values of rapeseed–cornmeal and rapeseed–soybean protein concentrate mixtures were similar to that of casein.

The largely beneficial results with mixed diets derived from plant protein suggest that the need to define further optimum complementary mixtures of plant proteins with each other and with fish and/or meat protein that approach the nutritional value of an ideal protein. For example, can the nutritional value of mixtures containing more than two protein sources be predicted from values for individual proteins?

Introduction of New Protein Foods. The successful introduction of a new food is a complex socioeconomic and scientific challenge described by Mockenberg et al. (1975) for Fortesan, a mixture of bulgur wheat, defatted soy flour, and skim milk used to feed malnourished Chilean children as a replacement of cow's milk. The authors state that a new product should (a) use readily available raw materials; (b) provide an adequate quantity of good-quality protein; (c) be consumed by the child and not be diverted to other uses; (d) be less expensive than the product it replaces; and (e) elevate the socioeconomic status of the consumer, i.e. it should not be patronized or designed to combat malnutrition, since no mother likes to admit that her child is malnourished. This strategy merits general adoption for the development and marketing of new foods.

Extensive field studies on possible nutritional benefits on feeding children lysine-fortified corn, rice, and wheat gave inconclusive results (Betschart, 1978, 1982; Latham, 1988). Since the dynamics of absorption, transport, and utilization of free amino acids such as lysine differ from those of protein-bound lysine, which has to be first liberated by digestion (Rerat, 1995; Schartner, 1989), lysine peptides might be better candidates for fortifying low-lysine foods than free lysine (Friedman and Finot, 1990; Sarwar and Paquet, 1989). Since peptides may also undergo less browning than free amino acids, possible benefits of peptide-fortified foods merit extensive study.

In addition to the usual growth and biochemical parameters, future studies should also address the improvement in health, general intelligence, and longevity of malnourished children consuming fortified foods and costs associated with the introduction of new foods (Caswell, 1991).

Genetic Approaches. Lysine, threonine, and tryptophan are nutritionally limiting in cereals as are methionine and cystine in legumes. Improved nutritional quality can therefore be achieved (a) by fortifying cereals with lysine or peptides of lysine which may undergo less browning than lysine during processing; (b) by fortifying legumes with methionine or methionine analogs and peptides which are less susceptible to change during processing and storage; and (c) through plant genetic techniques.

In principle, it should be possible to introduce new genes or to amplify existing ones in plants to modify the proportons of proteins that differ in amino acid composition. Such molecular approaches to enhancing the nutritive values of seeds are being intensively studied. Altenbach and Simpson (1990), de Lumen (1990), Falco et al. (1995), and Tabe et al. (1993) offer useful overviews of genetic approaches to improving the composition and nutritive value of cereals and legumes. Expectations are that these will lead to new transgenic plant sources for high-quality protein.

Germplasm collections, such as the USDA Collection of about 15 000 soybean seeds (Domagalski et al., 1992), could also serve as potential sources for the development of plant proteins high in lysine, methionine, threonine, and tryptophan via classical breeding methods and/or molecular biology approaches. Efforts should also be directed toward the production of high-quality proteins in plant tissue culture, bypassing the farm.

Removal of Antinutritional Compounds. As mentioned throughout the text, numerous naturally occurring food ingredients adversely affect the nutritional quality of foods. These include steroidal glycoalkaloids in potatoes, tomatoes, and eggplants; quinolizidine alkaloids in lupines; cyanogenetic glycosides in cassava; goitrogenic glycosides in cabbage; saponins and isoflavones (phytoestrogens) in fava beans and soybeans; protease inhibitors and lectins in legumes; chlorogenic acid in potatoes; ricin in castor beans; and others (Anantharaman and Finot, 1993; Deo and Friedman, 1994; Carnovale et al., 1991; Liener, 1994).

These compounds can be eliminated from the diet by processing and/or plant breeding and molecular biology techniques. Since thermal processing, often used to destroy antinutritional compounds, may also impair protein quality, future studies should emphasize suppression of plant enzymes that control the biosynthesis of undesirable compounds (Stapleton et al., 1991).

Browning Prevention. Enzymatic and nonenzymatic browning reactions of amino acids and proteins with carbohydrates, oxidized lipids, and oxidized phenols cause deterioration of food during storage and processing. The loss in nutritional quality and potentially in safety is attributed to destruction of essential amino acids, decrease in digestibility, inhibition of proteolytic and glycolytic enzymes, interaction with metal ions, and formation of antinutritional and toxic compounds. Studies in this area include influence of damage to essential amino acids on nutrition and food safety, nutritional damage as a function of processing conditions, and simultaneous formation of deleterious and beneficial compounds. These compounds include kidney-damaging Maillard reaction products, mutagens, carcinogens, antimutagens, antioxidants, antibiotics, and antiallergens (Friedman, 1995; Kingsley, 1995).

To develop rational approaches to minimize adverse consequences of browning reactions and optimize beneficial ones, studies are needed to relate compositional changes to nutritional and toxicological consequences. To catalyze progress, a need exists to define known chemical, biochemical, nutritional, and toxicological indices of browning and its prevention.

Effects of Proteins on the Immune System, Serum Lipids, and Receptor Sites. A need exists to explore beneficial and adverse effects of various protein sources on health. An example of benefits of adequate protein intake is illustrated by the apparent effects on the immune system. Bounous and Kongshavn (1989) evaluated the ability of about 20 protein diets to induce formation of immunoglobin M (IgM) plaque-forming cells in the spleen of mice. They found that lactalbumin induced the highest immune response. A lactalbumin diet also protected mice against infection by *Streptococcus pneumoniae* and dimethylhydrazine-induced colon cancer. Similar findings were observed with whey protein diets. Whey, the liquid residual byproduct of casein and cheese manufacturing, contains about 0.8% protein, mainly lactalbumin (Forsum, 1975).

The beneficial effect of lactalbumin and whey on the immune system is probably due to the fact that lactalbumin contains high levels of cysteine, which may induce the formation of supernormal levels of reduced glutathione (GSH) (Bounous and Kongshavn, 1989). The induced GSH may be responsible for the beneficial effects of lactalbumin on the immune system and the prevention of colon cancer (Friedman, 1994).

Gluten intolerance or celiac disease appears to be induced by peptides following digestion of wheat proteins (Kasarda, 1995). It is possible that rearranging the conformation of wheat proteins through sulfhydryl–disulfide interchange (Friedman, 1994) or other means will minimize the release of such peptides or enhance their susceptibility to digestion, thus preventing celiac disease. The same approach may ameliorate allergic responses to egg, milk, and soy proteins (Brandon et al., 1993; Burks et al., 1991; Cornell and Rivett, 1995; Djurtoft et al., 1991; Jost et al., 1991).

Kritchevsky (1989), Sanchez and Hubbard (1989), Szymczyk et al. (1995), and Yagasaki (1989) discuss mechanisms of hypo- and hypercholesterolemic effects of amino acids, peptides, and proteins. Although the observed effects appear to be dictated by the amino acid composition of the proteins producing active peptides on digestion, Anderson et al. (1995) suggested that the lowering effect of soy proteins on serum cholesterol appears to be due largely (about 70%) to the presence of phytoestrogens. Future studies should (a) define serum cholesterol; (b) develop amino acid composition–cholesterol-lowering relationships of various protein sources to help establish dietary guidelines; and (c) isolate and characterize the active peptides.

Partial proteolysis of casein releases opioid-active peptides that bind to receptor sites of various tissues, inducing analgesia and regulating hormone secretions (Meisel, 1989; Yoshikawa and Chiba, 1992). Other peptides derived from animal and plant proteins inhibit the angiotensin-converting enzyme (ACE), resulting in a decrease in blood pressure. Van Berenstein et al. (1994) showed that whey protein hydrolysates are hypoallergenic. Will mixtures of protein hydrolysates from several protein sources have multiple beneficial effects?

We are challenged to respond to these research needs.

CONCLUSIONS

This analysis of protein nutrition shows that plant proteins have an amino acid composition that is generally nutritionally less favorable than animal proteins. However, within each class there appears to be wide variation in nutritional value. Evidently, nutritional considerations were not nature's primary guide in the evolution of plant proteins. Current developments in plant breeding and molecular biology should catalyze revolutionary changes in the biosynthesis of more nutritious proteins. These include high-lysine cereals and high-methionine legumes. Development of improved protein sources to feed the growing population will be facilitated by parallel developments in our understanding of (a) factors governing the digestion, absorption, and utilization of amino acids from different protein sources; (b) protein–calorie nutrition and malnutrition; (c) adverse and beneficial effects of amino acid-fortified and complementary protein sources; and (d) the role of naturally occurring and processing-induced antinutritional and toxic compounds in plant and animal foods.

A need exists to avoid or alleviate shortages of high-quality and high-energy foods and feeds and thus counter or mitigate one of the more serious threats to the quality of life for ourselves and our descendants. Our objective should be to improve the quality, quantity, and safety of available food and feed sources by any and all feasible methods. Much more new chemistry and engineering is needed to support genetics and agronomy. Food fortification and supplementation need better guidance based on research. Deleterious reactions in food storage and processing need to be eliminated or minimized and beneficial ones optimized. New food sources need to be developed and beneficial effects of proteins and protein hydrolysates in human health need to be defined.

This essay distills and unifies widely scattered information to give the reader an indication of where progress is being made and where the emphasis should be placed to increase both the quality and quantity of food and feed proteins. The payoff will be better fed and healthier animals and humans (Brach and Bello, 1992; Kanwar et al., 1991; Willett, 1994).

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